WATER-REPELLENCY: HIGH HUMIDITY ORIGIN IN SOME SANDY SOILS

BY

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To the Memory of

Second Lieutenant Peter Paul Domiono

USMC

Born 18 October 1943, Industry New York. B.S., University of Virginia, 2 June 1965. Killed in action, Da Nang, Republic of Viet Nam, 1 July 1966.

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Water-repellency (WR) of several sandy soils is shown to increase dramatically at or near 100% relative humidity (100 RH). This mechanism of WR increase appears to be geographically wide-spread. Dilution experiments, antibiotic studies and scanning electron microscopy indicate a strong correlation of such WR increase with actinomycete growth.

Several experiments indicate that volatile materials are involved in WR increase at 100 RH. A volatile product of a bacterium living on a non-WR sand effectively halted increase of repellency in a WR sand.

Investigation of the chemical and physical properties of the WR-inducing material indicates that it is acidic and susceptible to attack by polar solvents. However, neutral water extraction, while reducing WR, does not appear to remove the inducing material. No evidence was found to indicate that the WR-inducing material exists as a coating over large portions of the sand grain surface.

The results of the experiments suggest a model for the rise and decline of WR in the field in which WR increases when the soil is at or 100 RH but declines either on wetting or desiccation at RH below 90.

INTRODUCTION

Soil scientists use the term "water-repellent" (WR) to describe the abnormal resistance of a soil to water penetration. Grieg-Smith (cited in Bond and Harris, 1963) suggested that microorganisms of unknown type were responsible for the development of water-repellency.

Water-repellency is common in areas of Florida, California, and Western Australia, characterized by sandy soils located in sub-tropical to Mediterranean type climates (Bond, 1969). However, water-repellent soils have been reported more recently in temperate climates (Reeder and Jurgensen, 1979; Miller and Wilkinson, 1977) and in non-sandy soils (McGhie and Posner, 1980) and may be considered global in distribution.

The WR phenomenon and its economic consequences remain poorly documented perhaps because it is not always recognized. Jamison (1945, 1946) believed that WR was a factor in the etiology of the citrus disease known as blight of sand-hill-decline. Osborn (1969) found that WR had a negative effect on the germination of certain grasses, especially on hill slopes. Bond (1972) found that WR strongly reduced barley yields.

The work reported here is devoted entirely to the origin and causal basis of WR in several sandy soils. We distinguish between cause and origin as follows: the

cause of WR is, presumably, an organic material which somehow hinders water penetration. This material can be expected to have a particular and therefore, identifiable, origin. It is usually thought to be a microbiological phenomenon. The origin may or may not be identical with the cause, depending on whether, for example, microorganisms themselves cause WR, or merely produce a substance which causes it.

The etiology of WR should be closely tied to the way in which WR increases and decreases under field conditions. Presumably, if we understand the origin and basis for WR, we should be able to correctly predict the changes in WR of soils.

The objectives of this research are to determine the origin of WR, to define its chemical cause, and relate these to WR as it occurs under field conditions

LITERATURE REVIEW

This review is restricted to that literature relevant to either the origin or the identity of the material causally associated with naturally occurring WR of soils.

Wander (1949) was perhaps the first to provide evidence that microorganisms are the origin of the WR material. He found that strong WR was associated with sandy soils receiving large amounts of Ca and Mg. He proposed that fatty acids produced by microorganisms reacted with the divalent ions to produce a hydrophobic soap that coated on the sand grains

Wander did not actually attempt to show that organisms in the sands produced significant amounts of fatty acids. Rather, his conclusion rests on chemical arguments for the existence of fatty acids in the soils and from there he reasons that these must be of microbial origin. What must be considered is the validity of Wander's conclusions as to the existence of significant amounts of fatty acids in his soils and their effectiveness in inducing WR.

Wander performed the following analysis.

> several composite soils samples which exhibited the water-repellent property were checked for the presence of fatty acids. This was done by heating the soil in the presence of a strong sodium hydroxide solution, neutralizing with sulphuric acid, and steam distilling in the presence of a slight excess of acid. A small amount of solid material

> was obtained which gave a test for a carboxylic acid group...
>
> A dilute ether solution of the solid material obtained from this steam distillation when added to the subsoil, which wet very easily, had no effect on wettability However, when a dilute aqueous solution of calcium or magnesium hydroxide was added to the soil, which had been previously treated with the ether extract, the soil became very water repellent when it was dry (1949, p. 299)

Wander's inference appears untenable in view of subsequent advances in humus chemistry. Sodium hydroxide randomly cleaves humic substances, with de novo formation of carboxyl groups. Wander might well have created fatty acid salts by this method, so long as there were humates on the sand to decompose (Hayes and Swift, 1978).

Wander could have met this objection had he repeated the fatty acid extraction on non-water-repellent (NWR) soil. Had the ether solution of this extraction failed to produce WR after Ca or Mg treatment, his results would have been more credible.

Wander also found that methanol extraction reduced WR and that putting the methanol extract back onto the extracted sand restored the lost repellency.

He then did the following. "When a portion of the methyl alcohol extract was evaporated to dryness, nitric and perchloric acids added for destruction of organic matter and heat applied, small globules of fat were released. Further heat oxidized the fat, and the residue, when taken up in distilled water, gave strong Mg and Ca tests" (1949, p. 299).

From this and the fact that Mg stearate is somewhat soluble in methanol, Wander concluded that what he had extracted with methanol was Mg or Ca stearate or some fatty acid salt closely related to them. On compelling reason for accepting this is that, when genuine stearic acid was evaporated onto NWR sand from ether and the sand then chased with "dilute" $Ca(OH)_2$, intense WR was created.

As striking as these results were, they are not entirely convincing. Wander does not demonstrate that his methanol extraction removes a stearate salt rather than some other form of Ca and Mg, which is methanol soluble. For example, $Ca(NO_3)_2$, which could be in the system, is methanol soluble; and $MgCl_2$ is slightly so.

In addition, Wander was not able to identify stearate in the methanol solution. Its existence was postulated from a report on the solubility of the divalent salts and the inference that soils must contain long-chain fatty acids because a harsh basic extraction yields a WR material.

Since Wander did not definitely prove that stearate was in his extract, it would be helpful to know if other members of its homologous series also induce WR. This would at least remove the necessity of claiming that stearic acid, in particular, is present in the WR soil.

Wander failed to do any microbiology and therefore may have overlooked the possibility that Ca and Mg may affect WR merely by providing special pH conditions favoring

WR-producing organisms, rather than by participating directly in creating hydrophobic compounds.

Accurate evaluation of Wander's findings is difficult because his descriptions of experimental procedures are not precise and complete. Acid, base, Ca, and Mg and stearate concentrations are invariably described as "strong, weak, dilute," etc. In reporting the methanol extraction, he does not state temperature conditions, nor how much methanol per gram of soil was used. The inadequate definition of experimental conditions makes it impossible properly to repeat his experiments.

Wander's conclusions rely heavily on the results of methanol extraction experiments. If methanol does reduce WR and if the extractate re-evaporated onto sand regenerates WR, then perhaps useful chemical studies of these extracts are feasible.

Bond and Harris (1963), working with coarse Australian sands, found that WR was always associated with high populations of fungi. Although the mycelial masses they collected in WR soils were complex in nature, they found that many hyphae exhibited clamp-connections, suggesting basidiomycetes. They hypothesized that the basidiomycetes actually produced a type of water-proofing material, but they noted, "it remains uncertain if basidiomycetes themselves are the sole, or even the main source of substances water-proofing the sand particles, because wherever they grow

there exists an abundance of hyphomycete fungi and bacteria" (1963, p. 120).

The work of Bond and Harris exemplifies the necessity of distinguishing between correlation and cause. Because basidiomycete growth correlates well with WR increase does not prove that it actually produces WR.

Bond and Harris did not attempt to induce WR in sands with organisms isolated from their sands. This may have been due to their inability to obtain pure cultures from the samples they collected.

Following the suggestion of Bond and Harris (1963) concerning the relation of WR to fungi, Bornemisza (1964) grew Aspergillus niger in broth, dried the mycelium, and applied it to dry ignited sand at rates of 2.0, 1.0, 0.5, and 0.125 % of soil weight. Before incubating the mixture, he obtained contact angles of 90, 89, 83, and 74 degrees by the capillary rise method (Emerson and Bond, 1963). King (1981) indicates that these values correspond to water-drop-penetration-times (WDPT) of 260, 142, 16, and 1s, respectively. The samples were then dry-incubated at 28C for 4 weeks. The only effect this produced was to reduce the first contact angle from 90 to 87 degrees (from 260 to 85s).

The first problem one sees with this experiment is the fungal mass needed to obtain significant WR. Some very WR materials have hardly this amount of organic matter in toto,

and far from all of that is fungal mass. Furthermore, Bornemisza produces no evidence to suggest that there is enough mycelium in natural WR materials to produce even these modest WDPTs.

The penetration times (PT) achieved by Bornemisza at 2% fungal mass are much smaller than those sometimes achieved in the field. Roberts and Carbon (1971) have found field PTs of up to 4800s.

Bornemisza seems to have been working with a different concept of the basis of WR than either Wander (1949) or Bond and Harris (1963). The latter workers considered WR to be due to a coating or "water-proofing" of the sand grain surface. The WR produced in Bornemisza's work seems to result from a simple mixture of sand and fungal mycelia. He presents no evidence to suggest that his incubation did anything to produce a coating. If the sand-mycelium mixture were to be sieved or "gold-panned," the light mycelium would almost certainly separate from the dense sand, leaving the latter NWR.

Savage et al. (1969b) inoculated spore suspensions of eight common soil fungi separately onto sand. Twenty mls of water suspension were mixed with 100 gm sand, so that the incubation was conducted at about twice field capacity (Grady, 1974). After 53 days of incubation, the air-dried sands inoculated with A. sydowi and P. nigricans

gave PTs of 17 and 16 seconds, respectively. The other organisms produced no repellency. Controls were sands with water and nutrient medium but no inoculum; these also produced no repellency.

Possible reasons for the low penetration times may include a) the low nutrient content of the suspending medium, which contained only glucose (0.5%) and glycine (0.13%) and may have been growth-limiting for some fungi; and, b) the water content of the inoculated sands, which may have been too high for good fungal growth.

In the same paper the authors reported that methanol and water extracts from the cultures, when air-dried on ignited sand, induced WR when the sand was heated. The extractions were done by shaking the sand with an equal weight of solvent for an hour and then decanting onto the sand. By heating the samples at 200C for 15 minutes, they were able to obtain very high repellencies for methanol extractions, less for water extractions. They achieved no water repellency with only air-drying.

In subsequent experiments, the authors tested the effects of three microbial humic acids and a microbial polysaccharide produced <u>in vitro</u> (Savage et al., 1969a). These dried materials were mixed with ignited sand at rates of 0.001, 0.05, and 0.1% and wetted with solutions of Ca, Na, Fe (III), and Al ions at various pHs. The mixtures were agitated and then air-dried and the repellency

tested. In a few of the systems, extreme repellency was generated but the authors conclude that the pH conditions under which this occurred were too unusual to be important in the soil.

Adhikari and Chakrabarti (1976) used microbial humic acids from S. atra and a Streptomyces sp. to make humate-Fe complexes. Mixing these dried materials with organic-matter-free soil at 1.4 rate, they achieved maximum penetration times of about 150s. No pH adjustments were made. The main problem here is that only moderate PT is induced by organic matter additions far above those found in sandy soils. Another is that, like Bornemisza, these authors have produced "extrinsic" WR; density separation would leave the heavier soil quite non-repellent. Many (perhaps most) cases are not like this, so the results leave those cases unexplained altogether.

Roberts and Carbon (1971, 1972) worked directly with the chemistry of natural WR material. They claimed to identify a 1 μm-thick organic coating on sand grains as the cause of the WR. The coating would be removed by wet-agitation or by treatment with Na_2CO_3. When the sand was soaked for 14 days in 0.005M Na_2CO_3, most of the organic matter was removed and the sand was left NWR. The solution was then separated into humic and fulvic acid fractions by the method of Kononova (1961). The fulvic fraction, when dried back onto the extracted sand, yielded no WR. The

humic acid fraction handled in the same manner re-induced WR. The authors imply that this humic skin is microbially produced and that, once formed, it is a permanent part of the "stable humic acid fraction" of the sand.

As suggestive as this experiment was, it was not conclusive in showing that the humic material was the source of the original repellency. The Na_2CO_3 extraction is unlikely to leave the humic acid complex unchanged with the result that the re-applied material may not be the same as that removed. Second, the authors do not state at what temperature the humic extract was re-dried on the cleaned sand. If this temperature was well above what could be expected in the field, then they may have created an artifact. Third, the original extractate contained both humic and fulvic acids. Presumably, so did the original sand. Since the fulvic acid is NWR, there is no reason to believe that the original humic/fulvic mixture on the sand was WR. Perhaps the original repellency was due to some other factor, such as acidic polysaccharides, also removed by the extraction, but not re-precipitated back onto the sand in its original form so that it failed to re-induce WR.

Roberts and Carbon also studied humidity effects in WR sands. These experiments were designed to explain how WR "appears during the last intermittent rains of spring (October), but disappears during the following rainy winter" (1971, p. 19).

After drying the sand at 50C, the authors placed pre-wetted and non-pre-wetted samples at humidities ranging from 90 to 100% relative humidity. The penetration time for all samples before wetting was 600 s. Initial PTs were 0 and 600 s for the pre-wetted and non-pre-wetted samples, respectively. On incubation at 100 RH for 2 weeks, the PT of the non-pre-wetted samples declined to <1 s. At lower RHs, these samples retained PT = 600 s. The pre-wetted samples remained at PT <1 s when incubated at humidites ≥97 RH. At lower RHs, the PTs of the pre-wetted samples increased on incubation For example, a pre-wetted sample incubated for 2 weeks at 90 RH had a PT of 40 s. After 3 weeks at this RH the PT increased to 50 s. (The experiment was terminated after 3 weeks).

The first problem is that the authors are not really working with a natural sample, but one which has been heated to 80C for an unstated length of time. This has an indeterminate effect on the results.

The pre-wetted series is supposed to show how WN due to a permanent but wettable coating might be regenerated on drying. At the lower RHs the coating simply dehydrates and some of the PT returns. But, in fact, the original high PT is not regained Are we to infer that at lower humidities and/or longer incubation times it would be? The data presented only hint at how high penetration times might be regained.

According to Chen and Schnitzer (1978), at soil pHs at or below neutrality, humic acids contribute to WR if they dominate the humic acid-fulvic acid system. If fulvic acids dominate, they will likely dissolve and improve wettability at these pHs. Both humic and fulvic acids contribute to soil wettability at basic pHs. The authors believe that this hypothesis explains the observations of Holzhey (1969), who found that areas in southern California become WR after autumn rains but became NWR after long periods of desiccation. Chen and Schnitzer (1978) believe that the increase in WR is due to leaching of fulvic acids in the rains, which leaves the WR-inducing humates behind.

Miller and Wilkinson (1977) and Wilkinson and Miller (1978) investigated the sandy soils of Ohio golf greens to determine the nature of the WR. They used scanning electron microscopy (SEM) to identify the coating on the grains as a combination of amorphous and mycelial organic material. They also found that sand grains from NWR areas showed no such covering. The coating material tended to aggregate the sand grains in the affected areas. The best defence against the repellency, they said, was to keep the green wet. Repellency increased with drying.

Wilkinson and Miller (1978) claimed to identify the WR material as a fulvic acid. The sand was extracted with 5% NaOH and purified by passing it through a Dowex 50 resin

acid column. The material was then freeze-dried and prepared for IR as a solid. The IR spectrum obtained was "almost identical" to those described by Stevenson and Goh (1971) for soil fulvic acids. They hypothesized that WR is due to the formation of Ca and Mg fulvates which become hydrophobic after a prolonged drying cycle.

With respect to the view that WR is a result of a Ca/Mg salt, they simply give no evidence for the claim. They did not analyze the fulvic extracts for these elements. Their work on the organic basic extract does not prove the point. They do not show that the NWR areas fail to yield extractates with the same IR spectrum. Unless this is done, there is no reason to think the chemistry of the WR is special. Their argument could have been strengthened had they induced WR with Ca or Mg precipitates of their extracts from WR areas.

The SEM study (Miller and Wilkinson, 1977) is more compelling. The photographs show a coating of remarkable density and thickness on WR sand grains.

Several investigators have observed seasonal variation in WR. Jamison (1946) found that wetting a WR soil temporarily cured the problem, but the resistant condition returned on drying; the point is reiterated by Wilkinson and Miller, 1978)

Bond reported that for south Australian sands, "At all sites the soil became markedly more repellent after

the first heavy rains or the autumn while the soil was still warm, and in the period when basidiomycetes were most active in growth. The repellence persisted through the winter, with some decrease in intensity, but the largest decrease occurred in the late spring and summer" (1969, p. 261).

Holsher observed that southern California soils with low litter contents were, ". .usually quite wettable when thoroughly desiccated in summer. However, following autumn rains, the moistened areas may rapidly become extremely repellent" (1969, p. 31).

Roberts and Carbon report on "...observations in the field, where water repellence appears during the last intermittent rains of spring (October) but only slowly disappears in the following rainy winter" (1971, p. 19). In direct reference to the mechanism of WR increase, they stated that "dehydration of these organic skins during the hot summer caused them to become hydrophobic and they did not readily rewet during the subsequent wet winter period. As a consequence a very uneven pattern of water infiltration and storage occurred in early winter" (1971, p. 13).

MATERIALS AND METHODS

The Samples Used

Five sample types were used in this study.

1) A St. Lucie sand (typic quartzsamment) from Lake Worth, Florida, labelled, L;

2) A Tavares sand (typic quartzsamment) from Bartow, Florida, labelled, H;

3) An Arredondo sand (grossarenic paleudult) from Gainesville, Florida, labelled, G;

4) A beach sand from Kauai, Hawaii (unclassified) labelled, B;

5) A Eustis sand (psammentic paleudult) from Gainesville, Florida, labelled, E.

All samples were taken from the top 15 cm of soil.

The L, H, and W sands exhibited intrinsic WR (IWR), in that, when dry-sieved so that the separable organic matter "floated" on the top of the sand, the samples remained WR. This indicated that the WR material was firmly attached to the grain surfaces in these cases. The carbon contents of these samples were quite low (C = 0.1% by induction furnace).

The G sand was substantially different. When sieving density-separated the particulate organic matter, the sand itself was NWR, in spite of the high WR of the original

asterial. The particulate organic matter separated in this way remained WR. This was designated extrinsic water-repellency (EWR), assuming that the sand itself was NWR. It had a carbon content of about 1%.

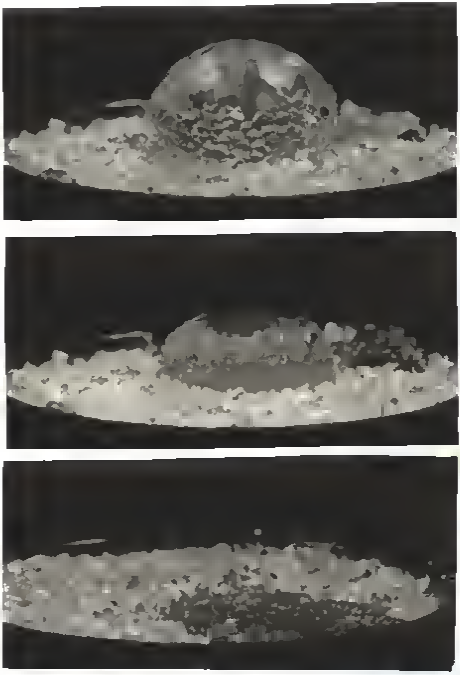
The Eustis (E) sand was not water-repellent. It was used for comparative purposes.

Method of Quantitating WR

We chose the water-drop-penetration-time (designated PT) method for quantitating WR. A small portion of sand (usually about 0.8 gm) was placed in the cap of a 3 ml vial. The cap was tapped several times on the work bench to obtain a uniform bulk density. A drop of deionized water (0.04 ml) was then placed on the surface with a microliter syringe. A stop watch was used to assure the time for the drop to completely penetrate the sand as observed under a dissecting microscope. Figure 1 shows the penetration of such a drop.

At least three PT measurements were made for each sample. Averages are reported here. For the basic experiments establishing the increase of PT at high relative humidities and its relationship to actinomycete growth, the differences between the values compared in the experiments were found to be significant at levels of 0.1 or better. In other experiments, particularly those relating to the effects of volatile materials on PT increase and

Fig. 1 Photo micrographs showing three stages of penetration of a water drop into WR L sand.



suppression, such levels of significance were not achieved for individual experiments. For this reason, several replications of such experiments are presented here. It would be useful to improve the quantitative quality of such experiments in the future.

For samples exhibiting unusually high PTs, PT measurements were stopped at 1200s.

There has been debate over the best method for quantitating WR (Letey, 1969; King, 1981). Some workers prefer capillary rise methods to PT (Yuan and Hammond, 1968; Emerson and Bond, 1963). There are a number of reasons for choosing the latter. Water-drop-penetration-time is much faster than capillary rise and uses much less sample. Since the work required thousands of measurements, PT was the only practicable technique. Penetration time measurement is also the most commonly used in current studies. Results from PT measurements are most easily compared with those of other workers. Finally, the investigations of Yuan and Hammond (1968) and King (1981) indicate that PT gives results comparable to those obtained by the capillary rise method.

The term "water-repellency" is a vague one, which does not imply a single defining experimental measure. Therefore, one is free to choose the most convenient measure so long as it is realized that it may not correspond exactly to the results of other measures. In the work that follows,

the terms "water-repellency" and "water-drop-penetration-time" are treated as synonyms.

Sample Handling and Selection

Time considerations dictated that most of the work be done using the L soil. The other samples were used primarily to test the universality of results of work first done with the L sand.

The L material was visibly cleaner than the other materials. This was a significant advantage in visible and electron microscopy, since there was little extraneous material to interfere with the observation of the surface organic materials. It also meant that stains gave good contrasts with the sand background.

Samples of uniform particle size were used in the PT experiments because they gave more reproducible PTs. The samples were separated into size fractions by shaking a sample through nested sieves (sizes 10, 20, 40, 60, and 100 mesh) on a mechanical shaker for 5 minutes. Sizes will be labelled here as, for example, 40/60 indicating the material passed through a 40-mesh sieve but not a 60-mesh sieve.

Usually no attempt was made to separate particulate organic matter from the soils used for PT experiments. Attempts to separate organic particulates from large sand samples failed because the manipulations involved in

separations invariably dislodged more organic materials from the sands. Such a separation was done for the differential-scanning-calorimetry experiment, where samples were small, and for which it was important to reduce extraneous organic material to a minimum.

Other Analytical Methods

Scanning electron microscopy (SEM) was done with a Hitachi™ model S-450. Samples from high humidity containers were air-dried prior to being gold-coated. No other treatment was found necessary.

Infrared spectra of solid organic materials from the sand surfaces were run on a Perkin-Elmer™ model 567 instrument using KBr pelletization.

X-ray diffractions of the clay on the sand surfaces were conducted using a General Electric™ model XRD 700. Organic matter and heavy metals were removed from the samples using standard methods (Whittig, 1965).

Total carbon analyses were conducted using a Leco™ induction furnace. Soluble carbon was run on the extractates of the L material with an O. I. Corp. model 504 total carbon analyzer. The ampuls technique was followed (Menzel and Escarro, 1964).

Calcium, Mg, Fe, and Al were done on neutral water, methanol, Kcl and pH = 14 (1 N NaOH) water extracts of the L sand with a Perkin-Elmer™ model 460 atomic absorption spectrophotometer.

Differential scanning calorimetry was done on the 40/60 sieve fraction of the natural L sand using a Dupont™ model 990 instrument calorimeter with gold pans. The calorimetric standard was ignited sand.

A number of solvent extractions were performed on the L sand, using 20 gm of 40/60 material poured into a 1 cm glass tube. Extractant was dripped onto the top of the tube such that, once the sand column became saturated with extractant, it remained so until the end of the procedure. Solvent was used at a 10:1 solvent to sand ratio (volume.weight). The solvents were water, ethanol, methanol, acetonitrile, and n-hexane.

Incubation Methods

At least one incubation of each sample was carried out in a dessiccator at 100 RH and room temperature in an air-conditioned laboratory (22C ≤ T ≤ 26C). Some samples were incubated at lower humidities. Desired dessiccator humidities were maintained using saturated inorganic salt solutions (Winston and Bates, 1960).

Time-course incubations were done by placing replicate samples in small petri dishes at the desired RH and removing them individually at specified times. A number of time-course studies were performed with antibiotic-treated sand see Table 1). This was done by soaking the sand in an antibiotic solution of the desired concentration and drying the

Table 1. Antibiotics used in PT increase incubations and their microbial suppressive activities.†

Antibiotic	Activity
aureomycin	general anti-microbial
chloramphenicol	general anti-bacterial
cycloheximide	general antifungal
2,4 dinitrophenol	general antimicrobial
malachite green	none
nystatin	general antifungal
penicillin	anti-gram positive bacterial
rose bengal	general anti-bacterial
streptomycin	anti-gram negative bacterial
sulfanilamide	anti-gram negative bacterial

†Source: Brock (1979).

sand-solution in a laminar flow hood. Controls for this purpose were the same sample soaked only in the solvent.

Concentrations of the antibiotics were chosen based upon Johnson and Curl (1972) and Williams and Davies (1965). The choice of concentration used in the sand system was made with two considerations in mind. First, the material would be dried onto the sand surface, and thus, the apparent concentration would be greatly magnified in comparison to solution concentration. Secondly, the sand contains considerable amounts of organic matter, which could absorb and perhaps deactivate the antibiotic. As a result, typical agar medium concentrations were adopted. Solutions were prepared at 50 and 30 mg/l and applied 1:1 to the sand (volume=weight). Cycloheximide was used at concentrations up to 200 mg/l as well as the more typical concentrations.

Nystatin is water-insoluble and was applied to the sand in ethanol solution at 50 mg/l The control sand was treated with reagent ethanol.

Microbiological Methods

Attempts were made to induce WR in NWR sands using pure cultures (or mixtures of them) of 26 organisms isolated from WR L sands. The isolates were obtained by agitating WR sands in a Waring™ blender at medium speed with 0.01 M NaCl (1.1 sand:saline) After a brief settling period (10 seconds) the saline suspension was plated on three selective

media. Media used were a) tryptic soy agar (TSA) plus rose bengal (50 mg/l) for bacteria, b) TSA plus streptomycin (50 mg/l) for fungi, and c) water agar for actinomycetes (Johnson and Curl, 1972). Distinctive colony types were re-streaked on the original medium until uniform colonies were obtained. These apparent pure cultures were then streaked onto slants for storage.

Two other isolation procedures were used based upon laboratory experience. Water-repellent sand was incubated adjacent to an ignited sand, being separated only by nylon mesh. Hyphae from the WR sand penetrated the nylon mesh and adhered to the ignited grains in "beads on a string" fashion. Individual hyphal fragments were cultured and inoculated onto test sand. In the second procedure, the number of isolates was reduced by selecting those that grew well at 100 RH. To do this, WR grains were fixed to microscope slides with collodion and incubated for 10 days (100 RH, room temperature). The slide was then held above a Petri dish containing TSA and tapped. Spores and/or hyphae of actively growing organisms, perhaps associated with the induction of WR, were dislodged and fell onto the plates, while those which had failed to grow under these conditions tended to remain on the sand.

The isolated organisms were used in inoculation experiments to try to induce WR onto ignited sands. To this end, the individual organisms were grown in American Type

culture Collection broth for several days. They were then centrifuged and washed with sterile saline. After several washings, the organisms were taken up in 50 ml deionized water. Three-tenths ml of this suspension was inoculated onto 3 gm of ignited sand in a serum vial, fitted with a cut-off syringe barrel filled with cotton. These latter were autoclaved prior to inoculation. The syringe-barrel assembly served to admit air to the system. One ml of ATCC food mixture was also injected. The inoculations were allowed to incubate at room temperature for a minimum of 1 month. Controls for the experiments were dead cells inoculated in the same way. At the end of the incubation period the sand was removed from the vials, dried at 100C, and tested for FT.

Several incubations were performed using γ-irradiated material. Soils were irradiated using a cobalt source placed 2 inches from 15 mm glass test tubes containing 4 gm of soil. Irradiation was performed for about 15 hrs, to give total radiation doses of $1.5\text{-}1.9 \times 10^6$ rads.

A series of dilution experiments were performed in order to follow the relationship of FT increase to microbial activities at 100 RH. Several Petri dishes, each containing 10 gm of L material were incubated at 100 RH and room temperature. Dishes were removed in pairs at specified times. One was used to test FT. The other was transferred to a Waring® blender containing 100 ml of sterile water and

agitated for 2 minutes at medium speed. From this suspension dilutions of 10^{-2}, 10^{-3}....10^{-7} were made. Each dilution was streaked in triplicate on the fungal, bacterial, and actinomycete selective media. The plates were incubated at 28C. The bacteria were counted after 24 hr, the fungi after 48 hr, and the actinomycetes after 72 hr.

Defined media were used to test the WR suppression effects of various organisms isolated from Eustis sand. Formulas for the salt, trace element, and vitamin solutions used in the media were those of Tisdje and Dazzo (1982). Glucose (0.25 gm/l) was used as the carbon source. Yeast extract (3.0 gm/l) was used as a vitamin source (Maynell and Meynell, 1965).

Anaerobic incubations were performed using L and B materials. Ten gram samples of sand in Petri dishes were placed over deoxygenated water in Gas-Pak 100 containers (Fisher Scientific Co.) containing a Pd catalyst for oxygen removal. A tube of resazurin indicator was placed in the system to indicate when all free oxygen had been removed. The container was then flushed for 5 minutes with nitrogen and sealed. Incubations were carried out for 10 days at room temperature.

RESULTS AND DISCUSSION

The Microbiology of Water-Repellency

In this section evidence is presented for the role of microorganisms in the rise of water-repellency at high humidities.

Water-Repellency Increases at 100 RH--A Biological Process?

Yuan and Hammond state that "time required for the water drop to penetrate the soil surface increased very sharply when the soil had been equilibrated in a desiccator at 95% or greater humidity" (1968, p. 58). The present work largely confirms their result, although it was found that WR grows dramatically only at RHs very near to 100%. The dependency of WR increase on high RH could imply microbial involvement. For this reason we studied the microbiology of the system.

Initial experiments were conducted at 100 RH for 10-day periods at room temperature. Table 2 shows that all naturally WR materials (Soils L, B, G, and B) sharply increased in PT during a 10-day incubation. The E sample, initially NWR, failed to gain any WR during the same period of incubation. Wet L sand, also NWR, failed to become so at 100 RH. The high humidity treatment does not create WR where none existed before.

Table 2. PT increases of various sandy soils incubated at 100 RH and room temperature for 10 days.

Soil	$PT_{initial}$	PT_{final}
	seconds	
L	32	416[a]
B	47	535[a]
G	453	1108[b]
B	347	1172[b]
E	0	0
28C (L)	32	564
3C (L)	32	44
wet L (1% B_2O)	0	0

[a]Differences between PT initial and PT final are significant at the 0.05 level.
[b]Difference significant at the 0.1 level.

Table 3. PT increases of L and B sands incubated anaerobically at 100 RH and room temperature for 10 days.

Rep no.	PT_{init}(L)	PT_{final}(L)	PT_{init}(B)	PT_{final}(B)
	seconds			
1	124	119	10	600
2	136	132	8	334
3	29	40	8	198

The fact that PT of samples from very different Florida locations (including the EWR soil G), and from Hawaii all responded positively indicates that this mechanism of PT increase is widespread.

The dramatic increase of PT at 100 RH suggests that a biological mechanism is operating. Other 10-day incubations support this. When samples of L material are incubated at 28C and 3C, respectively, the former increased more than its room-temperature counterpart, while the material left at 3C increased only very slightly (see Table 2).

The results of anaerobic incubation of the L and H materials also suggest a biological mechanism. On three replications of the experiment, the H soil increased consistently in PT, while the L soil usually declined in PT It is difficult to explain these results other than biologically Apparently the H material contains an anaerobic WR-producing organism and the L does not. The results also imply that there is more than one kind of WR-producing organism (see Table 3).

Table 2 shows the 10-day rise in PT of the extrinsic (EWR) sample, G. Under Materials and Methods, this material was described as being characterized by the fact that, when organic matter is density-separated from the sand on sieving, the denser sand proved not to be WR while the organic matter remained WR. The repellency of the sample seems to

be due entirely to the particulate organic matter. When the material was incubated, this remained true. The denser sand, when separated, remained at PT = 0. However, when this separated sand was incubated independently of its particulate organic matter for 10 days, it grew in penetration time at quite a normal rate. (For duplicate incubations of the separated sand, initial PTs were 0 and final PTs were 1200s.

How this occurs is not understood. A reasonable conjecture is that the organic particulates contain both a WR-producing and a WR-suppressing organism. The latter suppresses a sand-attached WR-producing organism. In the absence of this suppressing organism, the PT of the isolated sand grows normally at 100 RH.

Time-Course Studies of WR Increase at Various Relative Humidities

Time-course experiments were performed to show WR increase at 100 RH (room temperature). The results for the 20/40 and 40/60 sizes of L sand are shown in Figs. 2 and 3, respectively. The result for the Hawaii (H) material is shown in Fig. 4.

At 100 RH all the samples show strong and essentially monotonic growth in PT over the 30 days of incubation. At 97 RH, PT increase is quite restricted compared to that at 100 RH. (This is distinct from the result of Yuen and Hammond (1969), where strong PT increase occurred above 95 RH).

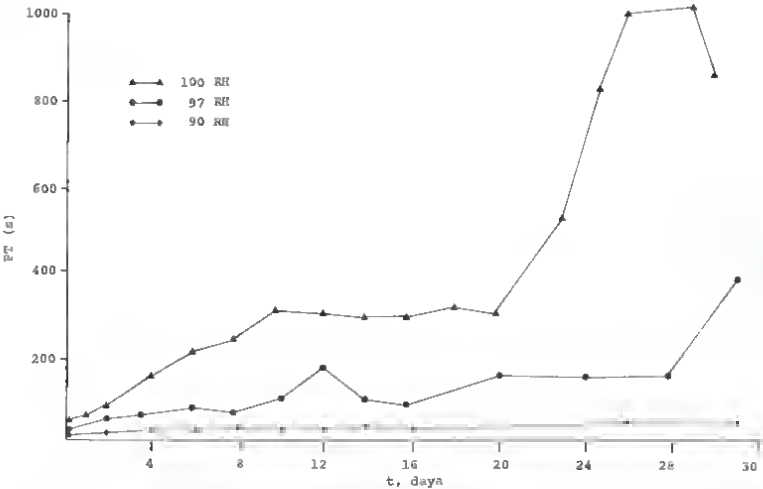


Fig. 2. PT versus time for 40/60 size L sand incubated at 100 RH and room temperature. After day 24, all differences in PT are significant at the 0.1 level.

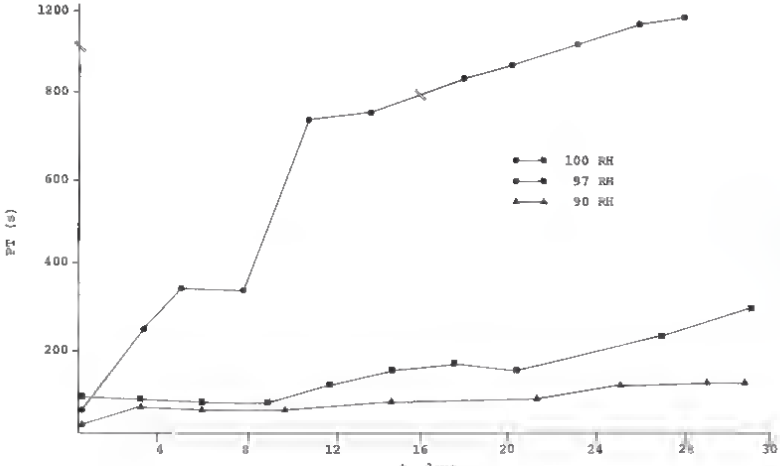


Fig. 3. PT versus time for 20/40 size L sand incubated at 100 RH and room temperature After day 10, all differences in PT are significant at the 0.1 level.

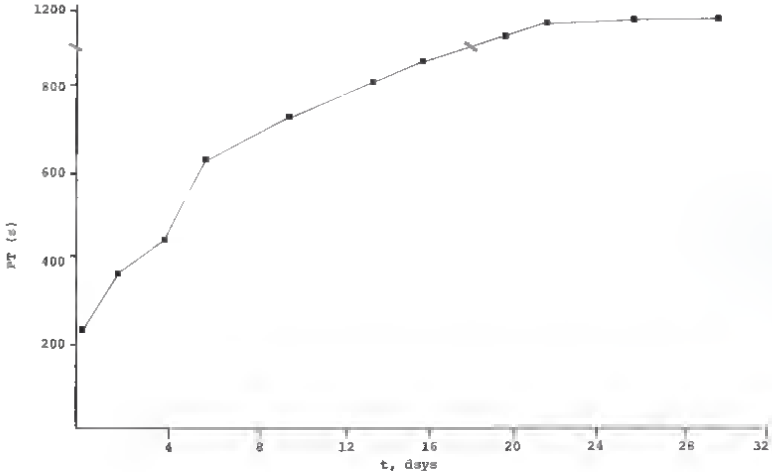


Fig. 4. PT versus time for M sand (unsieved) incubated at 100 RH and room temperature

At 90 RH growth in PT is non-existent in the 40/60 fractions and feeble in the 20/40 case. Lower humidity trials were not done for the H sand.

These results also could be attributed to microbial activity. The similar experiment of Roberts and Carbon (1971) produced the opposite effect. It may be that their soil system is dramatically different from those investigated here, or (which seems more likely) their 80C drying step before incubation did enough damage to the microbes that no WR could be produced.

Figures 2 and 3 also show that PT grows somewhat more rapidly for the 20/40 material than for the 40/60. In particular, the 20/40 sand does not show the lag period exhibited by the 40/60 between 10 and 20 days. The reason may be the different carbon contents of the two fractions. The 20/40 contains somewhat more particulate organic matter than does the 40/60 size as shown by the carbon analysis (0.3% C for the 20/40 fraction versus 0.1% C for the 40/60 fraction). That this is indeed the source of the more robust growth of the 20/40 fraction is indicated by the following experiment: Particulate organic matter was removed from the 20/40 fraction and the material finer than 40 mesh sieved out. The coarse organic material was layered on top of 40/60 sand in a 15-cm Petri dish at a 1:20 weight ratio. The control was the same amount of 40/60 sand without the organic matter. After 10 days

incubation, the organic matter was sieved away from the sand and the latter checked for PT. Table 4 shows the results, which confirm that the origin of the high rate of PT growth in the 20/40 fraction is the greater amount of organic matter present.

Figures 2 and 3 also point out that the PT of a sample is independent of the relative sizes of the individual pores in the sand. The pore size of the 20/40 material must, on average, be significantly larger than that of the 40/60 material. One would expect that PT would grow more slowly in this size fraction and probably not reach the same magnitude. Yet, at the end of a 30-day incubation, the PT is as high for 20/40 as for 40/60 and became so more quickly. The ultimate PT a soil might attain is unknown, and this could be higher for the 40/60 sand. However, the greatest PTs measured (about 4800s) are attained by both size fractions.

The Effects of Irradiation, Heat, and Antibiotics on Time Course Increase of WR

Figure 5 shows the effects of γ-irradiation (1.7 x 10^6 rads) and 80C dry-heat treatment (over night) on the L material. Two γ-irradiated samples were used. One was tested for PT four times in a 30-day period, the other only once at the end of 60 days. The γ-irradiated samples increased only slightly over the two incubation periods. The heat-treated material showed less PT increase than control, but not nearly so much as the γ-irradiated material.

Table 4. Organic matter enhancement of PT growth for incubation at 100 RH and room temperature for 10 days.

Rep. no.	PT (+OM)	PT (-OM)
1	363	294
2	385	167

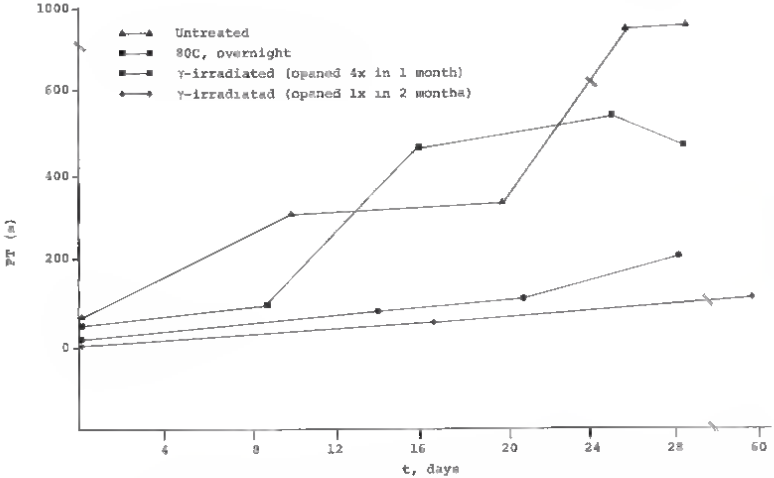


Fig. 5. PT increase of heated and γ-irradiated L seeds incubated at 100 RH and room temperature for 30 days. After day 20 PT differences between γ-irradiated material and untreated material was significant at the 0.1 level.

The restraining effect on PT increase of the 80C treatment may explain the difference between the results of out 100 RH incubation and those of Roberts and Carbon (1971). After heat-treatment at 80C, their sands declined in PT at 100 RH, whereas ours increased in PT, although at a reduced rate. If the organisms on their sand were more sensitive to heat than ours, the heat treatment may have destroyed all potential for PT growth. Their result at 100 RH may be an artifact.

Figures 6 and 7 show the effects of various antibiotics on the growth of PT over 30 days. Chloramphenicol, streptomycin and 2,4 DNP were the most effective agents. They completely eliminated PT growth for the entire incubation period at the 50 mg/l rate. Since the first two of these are generally believed to have no effect on fungi (Pestka, 1975; Schlesing and Medoff, 1975), it appears that PT growth is dependent upon prokaryotic activity. However, both cycloheximide and nystatin are effective PT-increase inhibitors. Nystatin is moderately so at 50 mg/l. Cycloheximide is completely so at 200 mg/l and has a moderate effect at 50 mg/l. Figures 6 and 7 together suggest that unrestricted PT growth at 100 RH is a result of both prokaryotic and eukaryotic activity.

Not all the antibiotics used are reported in Figs. 6 and 7. Aursomycin had no effect on PT growth. Malachite green is not an antibiotic. It was used because it

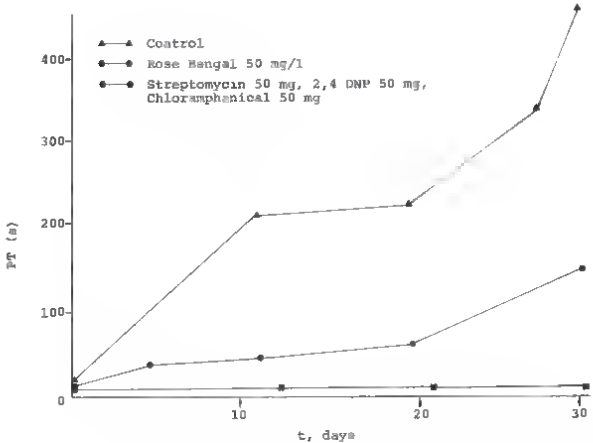


Fig. 6. PT increase of antibiotically treated L sanda incubated at 100 RH and room temperature for 30 days. Differences between control and other values significant at > 0.1 level.

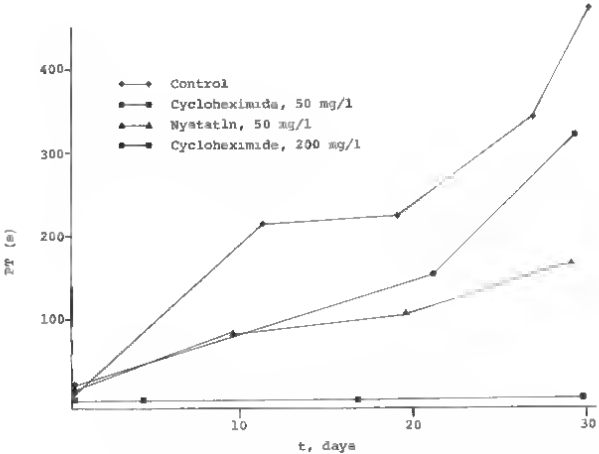


Fig. 7. PT increase of anti-fungally treated L sands on incubation at 100 RH and room temperature for 30 days. Difference between control and 200 mg/l cycloheximide significant at 0.05 level.

it adhered to the sand surface. As such it gives some evidence as to whether the antibiotics applied are truly affecting the microbes or simply having a surface-chemical effect. It had no effect on PT growth. The negative results from aureomycin and malachite green suggest that the other antibiotics are acting biologically.

Sulfanilimide and penicillin had only moderate effects on PT increase and are not included in the figures.

Dilution Experiments Correlate Microbial Populations with NR Increase

The observed effects of certain antibiotics on PT increase suggested the need to study population changes of the various microbial types with time. Dilution studies were carried out for 17 days at 100 and 90 RH. The results are shown in Table 5. Figures 8 and 9 show the correlations as plots of CFUs and one-half of PT.

PT increase with time follows the increase in actinomycete population most closely. The bacteria grow more in numbers at lower humidity. This may indicate that they are the best competitors of the three groups at 90 RH. It strongly suggests that bacteria and PT are not related--at least in a positive way. The fungi multiply the same amount at both humidities, suggesting that PT increase is not strongly associated with fungal growth. Although PT is not related to fungal numbers, some antifungal agents have a negative effect on PT increase with time. They may influence PT indirectly by providing vitamins needed by the

Table 5. Numbers of colonies (CFU)† present on L sand after various periods of incubation as determined by dilution experiments.

Days	PT	CFU x 10^{-6}†		
	(s)	fungi	bacteria	actinomycetes
		100 RH		
0	10	0.5	6.0	1.7
4	76	5.4	11.7	6.8
10	113	8.0	54.0	53.0
17	203	11.7	77.4	150.0
		90 RH		
0	14	0.2	12.8	2.9
4	37	3.8	53.1	3.7
17	49	4.6	112.1	7.9

†CFU = colony forming units.

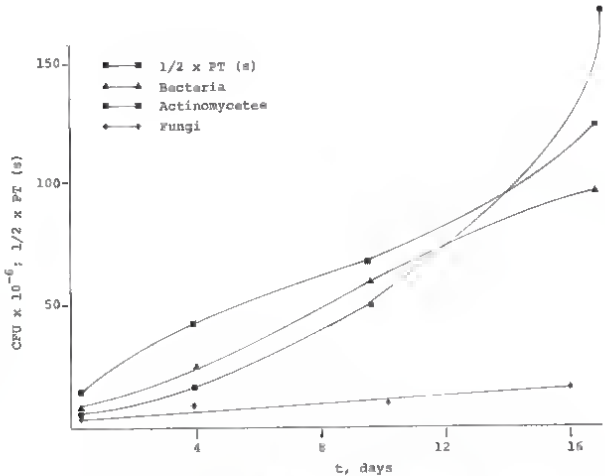


Fig. 8. Time-course increases in colony forming units and 1/2 x PT at 100 RH as indicated by dilution experiments using L soil. Difference between fungal and actinomycete CFU significant at the 0.1 level.

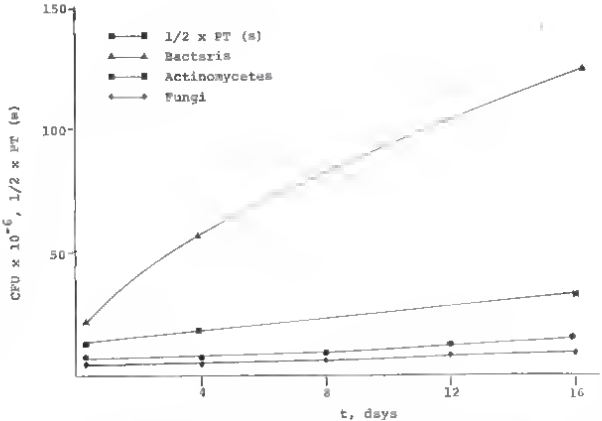


Fig. 9. Time-course increase of CFUs and PT at 90 RH by dilution experiments using L soil. Difference between bactsrisl and other CFUs significant at the 0.05 lsvel.

prokaryotes which affect PT directly. There is evidence that they play this role elsewhere in nature (Pramer and Schmidt, 1964).

Scanning Electron Microscopy Confirms the Role of Actinomycetes in WR Increase at 100 RH

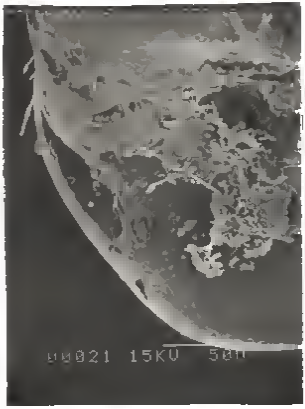
Scanning electron microscopy (SEM) also indicated the strong correlation of actinomycete growth to increase in PT at 100 RH.

The SEM revealed no organisms on the surface of NWR or natural WR sands. This was also true of incubated samples with very high PTs. Occasionally old, broken sporangia or hyphae were seen but even these were rare. It was concluded that sample handling might remove organisms from the sand surface before gold-coating. To avoid this, samples were placed on stubs with collodion and then the whole assembly was incubated at 100 RH prior to SEM observation. The results for various times of incubation are shown in Fig. 10.

The organisms shown were not simply growing on the collodion as a substrate. Actinomycete inoculum placed on collodion-treated stubs and incubated at 100 RH failed to grow

The surface growth in Fig. 10 is dominated by filamentous organisms with very small diameters and tight hyphal coiling typical of actinomycetes (Fig. 11). When

Fig. 10. Electron micrographs of L sands after incubation at 100 RH and room temperature for 3 (above) and 8 (below) days, respectively.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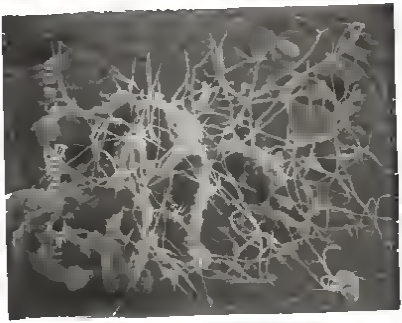


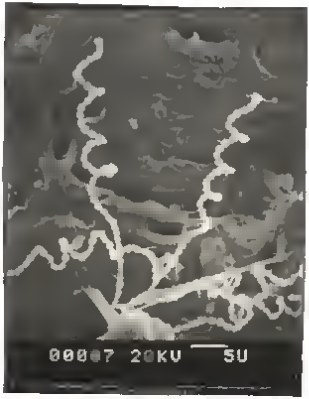


Fig. 11. SEM hyphae of actinomycetes on L sand exhibiting the tight coiling habit x 2900.

similar stubs were tapped over water agar plates, a rich growth of actinomycetes was obtained on incubating at 28C indicating that the rapidly proliferating organisms observed on L sand were indeed actinomycetes.

The Transfer of WR to Inert Materials After Incubation at 100 RH

Attempts were made to use 100 RH incubation to transfer WR from natural sand to ignited and γ-irradiated sand. In part this was done to gain information concerning the mibility of the WR material. Also, if transfer to ignited sand succeeded, the WR material would then be present on an otherwise chemically clean material, so that chemical extraction and analysis could be performed without excessive interference from irrelevant compounds.

In one case 40-60 size L soil was mixed with ignited sand (20/40 size) and incubated for 30 days. On sieve-separating the mixture, it was found that the L material had gained somewhat in PT, but that the ignited sand had not gained at all. In a similar experiment, 40/60 L material was mixed with 20/40 γ-irradiated L material. It was hoped that, since this sand was both abiotic and closer to natural sand, it might pick up any transferred WR more readily than did the ignited sand. It did not do so, merely retaining the small PT it began with. No attempt to transfer WR succeeded.

Inducing WR on Inert Materials Through Microbial Inoculation

None of the 26 organisms inoculated onto ignited sand at high water contents (1.3 gm water to 8 gm sand) reproducibly produced WR. Usually the PT of the sand was zero. Microscopic investigation of the sand surface of these samples revealed that microbial growth was poor under conditions near saturation. This was especially striking for the filamentous organisms. Very few hyphae were found after the incubations and those that were found were stunted.

Our results in these experiments are not greatly different from those of Savage et al. (1969a). Both systems may have suffered from being incubated at excessive moisture levels.

Better results were obtained when organisms were incubated at 100 RH. This was done by placing sand, organisms, and substrate in sterile beakers and drying this mixture in a laminar flow hood. The beakers were then placed in sterile desiccators at 100 RH.

A filamentous organism was obtained by the procedure of tapping a slide over a nutrient agar dish. This organism was placed at 100 RH as described above along with several unioculated beakers containing only sand and substrate. This was done because preliminary experiments showed that spores would move from inoculated to un-inoculated material, often producing higher PTs in the un-inoculated sand.

Table 6 shows the results for a 2 month 100 RH incubation of this organism after drying at 100C. (When air-dried or dried at temperatures up to 70C, the sand had a PT of zero) As will be seen below, natural WR materials increase strikingly in PT at 100C; and it is reasonable to speculate that what produces PT with this inoculation is related to the high temperature repellency of the natural system. Figs. 12 a and 12 b show SEMs of the surfaces of the inoculated and un-inoculated sands incubated at 100 RH. The presence of spores is strikingly evident, especially on the un-inoculated sand. It is not known if the spores themselves are the source of the WR of the dried material. The photographs do not reveal the presence of a coating on the sand grains.

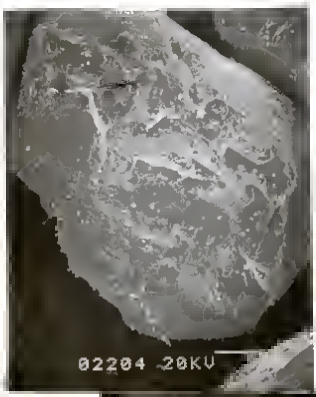
The Mechanism of the Decline of WR

The WR state is not a stable one, since it both rises and declines. If the rise of WR is due to microorganisms, how then does it decline? There appear to be two mechanisms. The rapid and dramatic one is wetting of the sand.

There is, however, a second mechanism of WR decline. It was observed that samples brought into the laboratory from the field gradually lost their repellency over several months.

Table 7 shows the WR decline of samples in the laboratory. Decreases in PT were usually not monotonic--

Fig. 12. a) Non-inoculated sand incubated in the same desiccator as sand inoculated with actinomycete. Electron photo micrograph shows a large number of spores on the grain surface. x 280; And b) spores on inoculated sand at lower density than on uninoculated sand. x 4000.


02204 20KV

2200 20KV 5U

Table 6. PT values of sands incubated at 100 RH with an inoculated organism.

Rep. no.	PT (+ inoc.)	PT (-inoc.)[b]	Control
	seconds		
1	64	200	0
2	83	193	0
3	72	155	0

[b]Differences significant at the 0.1 level.

Table 7. FT decline of various WR samples in the laboratory.

Sample	$FT_{initial}$	FT_{final}	Time elapsed
	seconds		days
L (with natural WR)	185	0	210
L (with WR derived at 100 RH)	3943	2400[b]	12
G	1200	550[a]	604
H	338	22[a]	129
E	102	57[b]	22

[a]Difference significant at the 0.05 level.
[b]Difference significant at the 0.1 level.

repellency occasionally increased--but the general trend was for PT to decrease with time.

Evidence from two types of experiments suggests that this decline occurs via a non-biological mechanism.

In the first type of experiment, homogenized samples of L soil were placed at RH values ranging from 90 to 0 using the salt solutions of Winston and Bates (1960).

One set of samples was removed from the desiccators after 30 days while the others were allowed to remain for 75 days. The results are shown in Table 8. The highest rate of decline occurs at 40-50% humidity. But repellency also declines quite dramatically at zero RH. Since microbial activity is negligible at this humidity, it appears that the decline is not biological. This conclusion is supported by a second type of experiment using γ-irradiated material. In this experiment a sample of L material was divided and placed in septum-sealed test tubes. Half were irradiated (1.5×10^6 rads) and half were left non-irradiated. The irradiated treatment lowered the initial PT of the material from 685 to 485s. Sterile, cotton-filled syringes were inserted through the septa of the remaining tubes. The tubes were then left in the laboratory environment for 60 days. Table 8 shows the results for the averages of two runs of the experiment. The percent decreases of the two sample types is remarkably close. This also suggests that the decline in PT is by a mechanism that is not biological.

Table 8. Declines in PT of L sands at various RH values for two incubation periods.

	RH condition (%)					
	90	75	50	40	15	0
PT_{init}	PT_{final}					
			20 days			
1976	-	-	1071	-	-	1522
			75 days			
371	173	90	96	58[a]	102	100
841	247	110	140	66[a]	198	196

[a]Difference from control different at the 0.1 level.

Table 9. Decline of γ-irradiated and non-γ-irradiated L material at laboratory atmosphere over 60 days.

Sample	$PT_{initial}$	PT_{final}	% decline
	seconds		
Non-irradiated	685	276	59.7
Irradiated	458	168	63.3

The decline of WR by this low RH mechanism is easily reversed by placing the sample at 100 RH.

Given that the long-term decline of WR is non-biological, we can show that Wander's mechanism (1949) for WR is not operating here. If it were, then Ca-stearate treated ignited sand should decline as does the natural material. In fact it does not. After 150 days, samples with initial PTs 3600s retained those PTs.

The low humidity decline is also evidence against the view of Roberts and Carbon (1971) that WR is due to the stable humic acid fraction of the soil. In our materials it is clear that WR is <u>not</u> stable even at low humidities. Its relation to the stable fraction must, at most, be indirect.

Finally, our laboratory results are in agreement with the field observations of Molzhey (1969) which indicated that southern California sands declined markedly in WR after long periods of desiccation.

The Loss of WR on Wetting and its Regeneration on Drying

The foregoing data show that PT increases by biological means at 100 RH. This result is the opposite of that obtained by Roberts and Carbon (1971). However, the fact that they pre-dried their samples at 80C may have adversely effected the microflora and created a situation in which 100 RH conditions merely wetted the sand, giving

low PTs, rather than stimulated the microbes to produce WR. Our 100 RH time-course experiments (Fig. 4) showed that the 80C treatment did not eliminate or reverse PT growth, but severely attenuated it.

In their other experiment, Roberts and Carbon (1971) pre-wetted high PT (600s) sant to a 1% water content and then put it in a desiccator at 90 RH. Some PT came back in a couple of weeks, as their data show (p. 15). Roberts and Carbon believe that this is due to drying of the WR coating.

This work was repeated using two sands of initially high or low PT. Each of these samples was then divided in half and one half was dried over night at 80C. The other sample was not dried. The drying had no effect on the initial PT. All samples were then wetted to 1% moisture level. PT became zero for all samples after this treatment. The samples were then put at 90 RH for 14 days.

The results are shown in Table 10. The controls, which were neither dried nor wetted, behaved normally for a 90 RH system: The high PT material dropped sharply and the low one gained slightly. The high $PT_{initial}$ predried sample increased about as much as did the correlative sample of Robert and Carbon (1971). They explain this as the drying of the coating at 90 RH. The non-predried sample gained less in PT. Table 9 also shows that the very low $PT_{initial}$ sample gained about as much PT as did the high $PT_{initial}$ one. If the coat theory is valid, the differences in PTs

Table 10. PTs after drying of pre-wetted L sand at 90 RH (room temperature) for 14 days.

$PT_{init.}$	PT after wet	PT_{final} not pre-dried	PT_{final} pre-dried	$PT_{control}$
seconds				
505	0	13	42	183
5	0	38	36	17

of dry materials are explained as differences in the amount of coating on the grains of the two samples. But then why does the low $PT_{initial}$ material gain almost as much PT under the treatment as does the high $PT_{initial}$ material? The experiment indicates that what happens is not so much the drying of an existing coat as the generation de novo of the PT. This happens because, when dried at 90 RH, the wet sample necessarily passes through a period when it is near 100 RH. During this period microbial activity is high and reinduces some little PT before the sand dried further. When water contents at the sand surfaces drop well below 100 RH, activity is low and no further PT is induced.

The regeneration of WR at 90 RH appears to be a biological rather than a physical process.

Volatile Compounds are Involved in WR Generation at 100 RH

Experiments were performed to determine if volatile materials, produced during microbial proliferation, are involved in WR increase.

The initial experiments were parallel to the previously described attempts to transfer WR from high PT sands to ignited and γ-irradiated sands. In these latter experiments the WR and NWR materials were separated so that only vapors from the WR sand would contact the ignited or irradiated grain. Five grams of each sand were

placed between two sterile fiber filters, which passed air and water vapor, but not spores. Below this were placed 15 gm of L sand in a large Petri dish. This assemblage was incubated for 45 days. No increase in PT of the "target" sands was observed. Thus, the normal L sand had little effect on the irradiated or ignited sands.

More indirect experiments indicate the involvement of some type of volatile agent. In one experiment, the effect on various packing densities of sand grains on the growth of PT at room temperature and 100 RH was studied. Five grams of 40/60 L sand were placed in each of the following vessels:

Vessel	Inside diam. (cm)
large Petri dish	14.6
medium Petri dish	9.6
small Petri dish	4.7
30 ml beaker	3.2
10 ml beaker	2.1

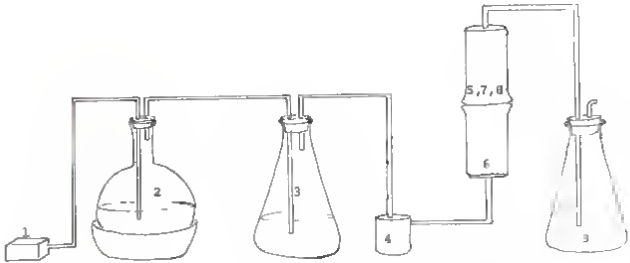
The various dishes form a sequence of increased packing of sand grains as the diameter decreases. In the large Petri dish there is virtually no grain-to-grain contact; in the beakers, almost all grains are surrounded by others. So, if a volatile is involved it is expected that the more densely packed material in the small containers would grow more quickly in PT because a volatile escaping from one grain would be more likely to adhere to

another (or itself) rather than escape. The results for three runs of the experiment are shown in Table 11. At the extremes of packing density, the increase of PT is higher for all three replicates having the denser packing. A volatile compound seems to be involved. If a volatile organic is involved in WR increase, it could be adsorbed on activated charcoal. A sample treated with charcoal should increase more slowly in PT than one which is not.

Activated charcoal (AC) (Fisher Co., no. 5690A) was sieved to obtain the 40/60 size fraction. This was mixed with 10 g of 20/40 size L sand in a 1:20 ratio. The control was an L sand without AC.

Table 12 shows that AC has consistently reduced the PT increase of the treated sand. This also indicates that a volatile in involved in WR growth.

The apparatus for a third experiment is shown in Fig. 13. It was designed so that air at 100 RH could be forced past a sample of 20/40 L sand. The control was the same apparatus without the air-flow system (i.e., the L material remained in stagnant air). If a volatile is involved than, to some extent, it is removed from the system by the air-flow. The system with the air-flow should give PTs less than those of the stagnant air system. Table 13 shows the results. In three of the five replications, the system behaved as expected and the control had a higher PT after 10 days than did the air-



1. Aquarium pump
2. 1-liter flask, maintains water at 70C
3. 1-liter flask, with water at room temp
4. Liquid trap
5. O-ring joint
6. Glass beads
7. Stainless steel screen
8. L 20/40 sample
9. 1-liter flask for back-pressure

Fig. 13. The 100 RH air-flow system.

Table 11. Variation in PT increase of L and with area^{-1} of container after 100 RH incubation.

Rep. no.	A^{-1} of container				
	.006	.014	.058	.125	.286
1	101	81	140	152	251
2	167	176	248	265	265
3	127	-	-	-	318

Table 12 Effect of activated charcoal treatment on PT increase on incubation at 100 RH and room temperature for 10 days.

Rep. no.	PT + Act. ch	PT - Act. ch.
	seconds	
1	264	689
2	122	411
3	129	380

Table 13. Effect of airflow on PT increase on incubation at 100 RH and room temperature for 10 days.

Rep. no.	PT + Airflow	PT - Airflow
	seconds	
1	79	200
2	72	169
3	65	113
4	44	32
5	70	61

flow system. However, in two cases neither the air-flow nor the control showed any significant PT growth in the 10 days. This phenomenon is unexplained.

A number of organisms isolated from the L sand produce noticeable odors on incubation at 28C. These odors can all be roughly described as geosmin-like. Plates of these organisms were placed in desiccators with L sand to see if the volatiles had any effect on PT growth. Control was an L sand with no plates of organisms. No effect was observed on PT growth over 10 days incubation.

The weight of evidence indicates that volatile compounds contribute to WR growth, as suggested by the grain-density and AC experiments. Whether the volatiles directly coat the grains or indirectly stimulate WR growth is not known.

<u>Eustis (E) Sand Suppresses WR Increase in L Sand. This Occurs via a Volatile Compound of Bacterial Origin</u>

The following series of experiments indicates that a volatile material is involved in the suppression of WR development in Eustis sand.

In the initial experiment, E sand of 40/60 size was mixed with L sand of 20/40 size. The two sands were then incubated together for 10 days at 100 RH and room temperature. At the end of the period, the two sands were sieve-separated. The results of this experiment and its replicates are shown in Table 14. The last column of the

Table 14. PTs of L (20/40) and E (40/60) sands sieve separated after incubation as a mixture (10 days, 100 RH, room temperature); PT of control incubated separately; PT of L (20/40) incubated independently after separation from E (40/60).

Rep. no.	PT, s			
	E (40/60)	L (20/40)	Control (L)	L (sep'd)
1	0	30	-	121
2	0	51	154	167
3	0	11	92	73
4	0	11	119	96

Table 15. Effect of sterile E (40/60) sand on PT increase in L (20/40) sand on incubation as a mixture (10 days, 100 RH, room temperature) followed by sieve separation

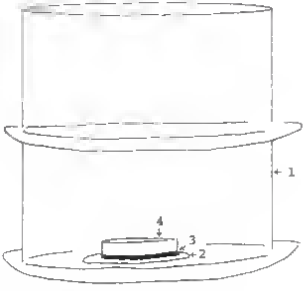
Rep. no.	PT, s		
	L, initial	L mixed w/E sand	control
1	7	76	80
2	8	92	82

table shows how the separated L sand behaved on incubation by itself. The table indicates that the Eustis sand not only failed to grow in PT, but suppressed the PT growth of the L material. This effect is transitory because, once the E sand was separated, the L sand grew in PT at a normal rate.

If the foregoing effect is biological then the experiment, repeated using sterile E sand, should show no repression. The control for this experiment was L sand plus ignited sand. Table 14 shows that the L material mixed with sterile E sand grew in PT as fast as did that of the L sand mixed with ignited sand.

In the next experiment, the E and L sands were separated from each other so that the L sand was incubated in the presence of whatever volatiles emanated from the E sand but had no direct contact with it. The system is shown in Fig. 14. The control was the same system with ignited sand replacing the E. The results are shown in Table 16. The PT increase of L sand was restrained compared to control, but not so dramatically as when there was intimate contact between the sand grains.

A transfer experiment was performed with the Eustis sand to isolate organisms proliferating at 100 RH. The E sand was incubated 10 days before the slide was tapped over the agar plates. Five apparently different organisms were isolated in this way--two filamentous and three non-



1. Plexiglss cylinder with nylon floor and 20 gm E or ignited sand
2. Stainless screen
3. 'O' ring
4. Small plexiglss cylinder with nylon floor containing 4 gm L 20/40 sand

Fig. 14. System for maintaining L sand at 100 RH in an atmosphere established by E sand.

Table 16 Suppression of PT increase of L sand by separated E sand on incubation at 100 RH, room temperature for 10 days.

Rep. no.	PT_{init},[a]		PT_{final},[a]		
	E	L	E	L	Control
1	0	10	0	102	172
2	0	12	0	88	130
3	0	10	0	80	161

filamentous. Table 17 shows that the pure cultures on TSB agar do suppress PT increase when closed plates are incubated at 100 RH with sand; the non-filamentous ones are more active than are the filamentous ones. The non-filamentous organisms were then grown on defined medium and the suppression experiment repeated. The effects of this are shown in Table 18.

The success of individual non-filamentous organisms, grown on defined medium, to suppress WR increase indicates that suppression occurs due to a volatile metabolic product of the organisms.

The Water-Repellent Coating

When low WR and naturally high WR L sand were compared under the electron microscope, no significant differences were found. There was no evidence of a coating on the grains of the highly WR material at any magnification. When the PT of L sand was raised to very high (4800s) levels at 100 RH, again no evidence of a coating was found. Figure 15 a and b show electron micrographs, at the same magnifications, of a low and very high PT L sand.

Objections may be raised to these negative results. It may be that the gold-coating treatment for the SEM destroys the organic layer before the sample is viewed. Or, it may be that the coating is too thin to be seen by

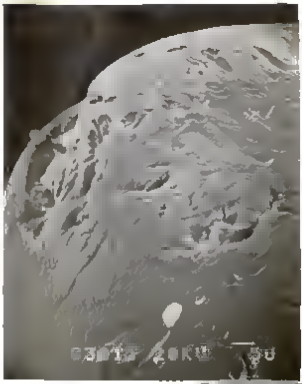
Table 17. Suppression of PT increase of L sand at 100 RH by TSB plates of organisms.

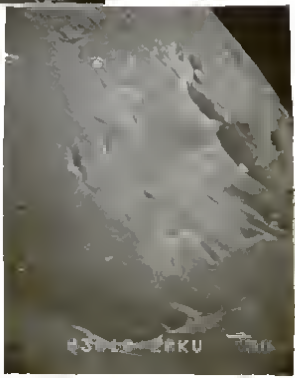
Rep.	$PT_{init.}$	PT_{final},[a]		
		filam	nonfilam	control
1	22	172	104	191

Table 18. Suppression of PT growth at 100 RH by defined medium plates of non-filamentous organisms.

Rep.	$PT_{init.}$	PT_{final} of named non-filamentous organisms			
		"round"	"spot"	"dot"	control
		seconds			
1	7	23	42	26	49
2	7	65	112	78	109

Fig. 15. a) Scanning electron micrograph of L sand sample incubated several months at 100 RH, room temperature. PT was approximately 4800s. No coating is evident on the grain. x 1200.
b) Scanning electron micrograph of L sand with low PT from laboratory. The surface is indistinguishable from that of the high PT material (a). x 1200.





SEM. The former is possible, but unlikely, because gold-coated samples routinely reveal extremely fine biological features. The latter objection may be valid. It should be noted, however, that if the coating is this thin, it is dramatically different from the comparatively thick organic layers which Roberts and Carbon (1972) and Wilkinson and Miller (1978) claim to see in their studies.

Roberts and Carbon (1972) were able to observe their coating under light microscopy when a drop of water was placed on the grain. As the drop penetrated the coating, a noticeable wrinkling of the coating material was observed. In a similar experiment, small drops of water were placed on individual grains. This was done by means of a 100 µl pipette drawn out over a flame and attached to a fine capillary. Water was drawn into and expelled from the pipette by mouth. By this method a drop very much smaller than the diameter of the sand grains was created.

No effect was produced on the grain surface that could not be ascribed to the optical distortions caused by evaporation of the drops. The drops evaporated rapidly under the microscope. Once the drop had evaporated, the grain surface appeared to be unchanged.

A second type of SEM study was undertaken in hopes that the coating might be seen "end on" even if it was invisible to the usual surface view. If the 20/40 size

material was lightly crushed in a mortar and sieved so that the 40/60 material was retained, while this material was initially very low in WR, some of the WR did return on incubation at 100 RH. However, the final PT was well below that of the original 20/40 material and that of natural 40/60 which had been raised in PT at 100 RH. An interpretation is that, while the original weathered surfaces of the cracked material gain in WR, the fresh surfaces exposed by the cracking do not. (That is, they seem to gain no coating.) It should be possible to see the edge where the old WR surface meets the NWR new one, and to see indications of the coating at this edge.

Figure 16 shows the edge between an old and a fresh surface after incubation. No coating is visible at the edge.

Etching the silica surface with HF vapor was also attempted. The idea was to find via SEM where etching had failed to occur because of an organic coating. 1 grains of 20/40 size were placed in small plastic caps. These were then placed in plastic Petri dishes containing several drops of HF. Each Petri dish was closed and kept in a fume hood until removed for inspection. On removal, the grains were rinsed with 95% ethanol and placed on SEM stubs. Samples were allowed to remain in the HF atmosphere for 12, 24, 48, and 72 hours. The 48 and 72 hour materials were almost completely dissolved by the HF vapor,

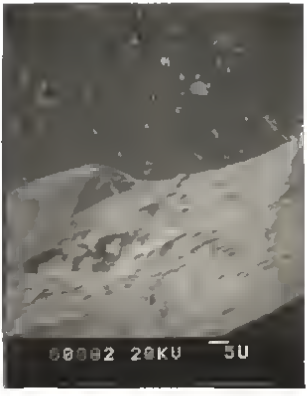


Fig. 16. The line between a freshly broken sand face (below) and the original weathered surface (above) of a "cracked" L sand grain. x1100.

so only the 12 and 24 hour materiele were actuelly rinsed and mounted. Figure 17 ehows the etching pattern obtained in thie way. Although the pattern ie not uniform over the surface of the grains, no pettern was found which could not be aecribed to the effects of oryetelline differences rether than to e coeting on the grain.

Fluoreecence microscopy wee eleo used. A laser exciting beam was direoted et e particular locetion on e grein as determined by e microecope coordineted with the beam. Grains of L sand were excited et 434 nm and reed at 536 nm. The obvioue epote of orgeniic metter on the grain eurfece fluoresced very etrongly. Very fine hyphee also geve dietinct peake. The bulk of the grain eurfece, however, caused no fluoreecence. If there were e coeting of eignificant thickness over the grein, one would expect that eome of ite component compounde would fluoreece. Thie experiment also indicetee the great thinness or non-exietence of e coeting over the majority of the grain eurfece.

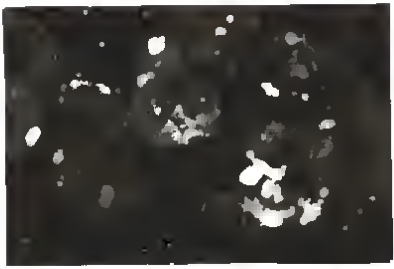
A eecond experiment eubjected L greine to ordinery fluoreecence microecopy. In thiee photogrephe (Fig. 18) the fluoreecing areas eppear white, while the non-fluoreecing ereas are green. Most of the eurfece feils to fluoresce, the white color ie confieed to eneil ereee on the eurfece. There appeered to be no difference between the fluoresoence micrographe of high WR and low WR eurfacee.

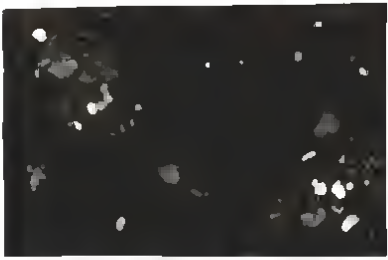
Fig. 17. a) Scanning electron micrograph of L sand grain etched with HF vapor for 12 hours. x 400.
b) Scanning electron micrograph of the same L sand grain at higher magnification. x 750.

00002 20KV 50U

00003 20KV 5U

Fig. 18. a) Fluorescence micrograph of highly WR L sand grains. White areas are particulate organic materials, which fluoresce. x 60.
b) Fluorescence micrograph of low WR L grain. x 60.





A number of stains were used to try to make visible any "coating." Only malachite green and Congo red gave positive results. But these stained only in the immediate neighborhood of the distinct organic spots on the surface The stains did not distinguish between NWR and highly WR sands

None of the foregoing experiments expressly denies the existence of a coating over most of the sand grain of WR material. It only indicates that, if it exists, it must be rather thin and chemically unresponsive A different type of experiment suggests there may be a coat.

This really consists of two experiments. In the first, plain glass beads (70 plus 140 mesh diam , Cataphote Crop.) were mixed in various percentages with beads of the same size which were silicone coated and extremely WR. The various mixtures were tested for PT. The results are shown in Fig. 19. Little PT is observed until the percent coverage reaches 30% and high WR is achieved only above 60% covered beads. This means that the total surface the water drop "sees" in penetrating the surface is, on average, 60% completely WR in the high PT case. So, of the total surface "seen," a quite large proportion is covered.

A similar experiment was run with naturally WR material (also Fig. 19). Here highly WR L sand was mixed with NWR L that had been extracted with NaOH to remove its WR. The PT versus percentage-coated-material curve is

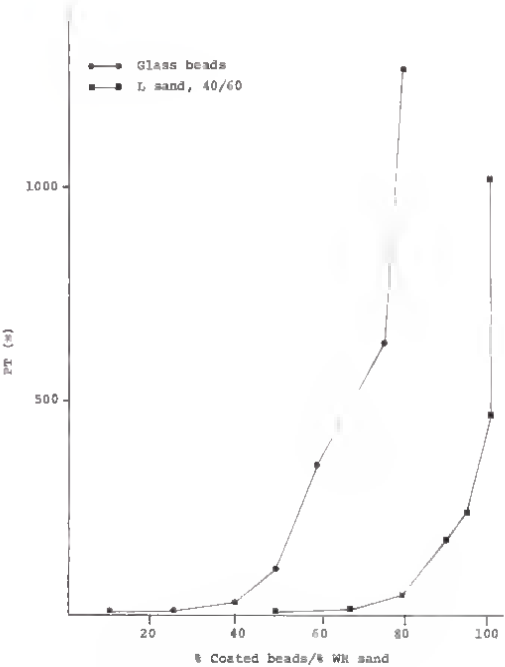


Fig. 19 PT versus percent coated beads and PT versus percent WR L sand.

strikingly similar in shape to that obtained with the artificial material. This is consistent with the idea that the mechanisms of resistance to penetration are similar in the two cases. The difference in the points where PT begins to appear along the "percent-coated-material" line can be explained simply by the fact that the naturally WR material is not completely covered as is the artificial material.

It is tempting to infer that the cases are quite similar and that the natural material is coated much like the artificial material. But we know nothing which says that this form of PT curve can only be obtained as a function of total percent coverage of the grains. It is entirely possible, for example, that the WR of the natural material is a result of rather isolated resistant spots, which, nevertheless, give a curve like Fig. 19 when such grains are mixed with various percentages of NWR grains.

The Chemistry of a Water-Repellent Material

Almost all of the work reported in this section was performed using the Lake Worth material. On occasion, other soils were used for comparison.

Some Basic Properties of the L Material

X-ray diffraction spectra were obtained for the solid material removed from the L sand surface by strong agitation. The identifiable materials were kaolinite, quartz, and amorphous materials.

Infrared spectra were also obtained for the surface material. The pattern seems to be unexceptionally close in appearance to spectra obtained from other Florida sources (Volk and Schnitzer, 1973).

The pHs of the various samples were run in the naturally WR, declined (low WR), and induced WR states (achieved by incubation at 100 RH). In all cases $5.8 \leq pH \leq 7.2$. No pattern among the three states was discernible.

The Aqueous Extraction Chemistry of L Sand

L sands of both 20/40 and 40/60 size were extracted at various pHs, then washed with neutral water, and dried at 70C. Figure 20 shows a typical PT versus pH-of-extraction pattern. The dotted line in the figure represents the original PT of the sand.

The affect on the penetration time of extraction near neutrality is consistent with Jamison's (1945) finding that water extraction reduces WR.[1]

At pHs greater than neutrality PTs decline only slightly more than those at neutrality, when dried at 70C. On this evidence alone, the belief that the WR material is acidic in nature, and therefore especially subject to

---

[1]Wander (1945) found that neutral water extraction did not affect WR. We shall see that drying a neutrally extracted sample at 100C increases PT above the original value. It may be that Wander dried his soils at 100 or more degrees and thereby re-induced WR.

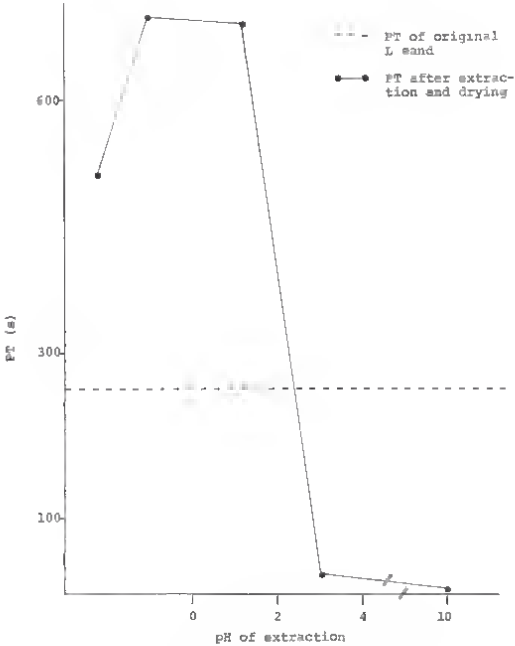


Fig 20 PT of L sand after extraction (10/1; v/w) at various pH values and drying at 70C.

high pH extraction would seem doubtful. Evidence that the WR material is in fact especially subject to high pH extraction will be adduced in a later experiment.

The anomaly in the curve of Fig. 20 is the increase in PT on low pH extraction and drying It is not possible to point to a single physical basis for this effect. If one assumes that WR is due to a coating of acidic material attached to the grain surface by hydrogen bonding, then an addition of protons may act by increasing the degree of hydrogen bonding. On the other hand, the acid may simply act to hydrolyze the organic material on the sand surface, which is then re-precipitated as a WR coat on drying. The SEM revealed that the sand surface of the acid-extracted material was not substantially different from that of the original material.

Although it is not possible to specify a particular mechanism for the result, it is useful to know that WR is not destroyed, but enhanced by acid treatment.

Above pH = 3 the PTs decline with extraction and drying. Until the initial extractant pH reaches about 11, no color is seen in the effluent. At pH = 11 and above, one sees an increasingly dark humate color. This leads one to wonder if the WR material is actually being extracted at pHs near neutrality, or whether the solvent is simply hydrating the material

Direct chemical evidence on this point is equivocal. An HPLC scan of the pH = 7 extractate which had been preconcentrated times 10, failed to show <u>any</u> peaks at 254 nm. This indicates that there is less 0.1 ppm of 254 nm-sensitive material in the extractate. There may, of course, be other 254-insensitive organic compounds in the extractate, but this surprisingly low figure is consistent with the belief the WR material is not being removed.

Soluble carbon assays were done using three types of sample. L sand directly from the field, of moderate PT, high PT material created at 100 RH in the L sand; and an L material whose PTs had declined in the laboratory. Three replicates were done on three samples of each type. Table 19 shows that more material was consistently extracted from the low WR sand, but the assay does not reveal anything remarkable about the high WR material.

Indirect evidence does, however, suggest that the WR material is not being removed in a neutral extraction. A number of WR materials, including the L, were placed in the caps of 1/4 ounce vials. To the surfaces of these sands one 0.04 ml drop of neutral water was applied. As Table 18 shows, this single drop had a powerful reducing effect upon the PT when the affected sand was dried at 70C. On the other hand, when these sands were extracted with water at a 10/1 rate, the effect was no greater,

Table 19. Soluble carbon contents of neutral water extracts of L sands (10/1.v/w)

Sample	$PT_{initial}$	PT_{final}	C content†
	seconds		g/ml
field	141	18[a]	3.58
high WR (100 RH)	1200	3[a]	6.13
low WR (lab, decline)	6	4	8.54

†The number reported here is an average of three replications.
[a]Difference significant at the 0.1 level.

after drying, than that of a single drop. This suggests that the latter method did not actually remove the WR material; in this pH area the water serves only to hydrate the WR material (see Table 20).

The results from extraction with water at near neutrality indicate that once a WR field soil is wetted, it loses its repellency. Water-repellency workers have held long-term drying may serve to re-induce WR after it is lost. The view is explicit in Roberts and Carbon (1971) who refer to WR being due to dehydration of organic skins over the hot summer. Similar views are expressed less clearly by Wilkinson and Miller (1978).

Our results do not support this view. We first dried a number of low WR sands, which were not pre-wetted, at 40 and 60C for 7 days. Figure 23 shows that virtually no increase in WR occurred over the period. Since the latter temperatures are well above those in the field, it does not appear that WR is restored by drying.

L sand which had an initial average PT of 94s was wetted to the 1% moisture level. At 1, 5, 14, and 30 days drying at 70C, the PTs were: 4, 6, 7, and 5 seconds, respectively. H material of 300s initial PT, treated in the same way, had final PTs of 11, 15, and 10s, respectively. Simple drying does not significantly raise PT.

On the other hand, if a water-extracted L sample is dried for 4 hours at 100C, more than the original repellency

Table 20. Decrease in PTs due to single drops of water and 10:1 extractions.

Sample	$PT_{init.}$	PT_{final} (single drop)	PT_{final} (10:1 extr.)
E	161	8[a]	5
H	338	10[a]	33
L (natural WR)	415	11[a]	20
L (high WR, 100 RH)	1200	26[a]	18

[a]Difference significant at 0.1 level.

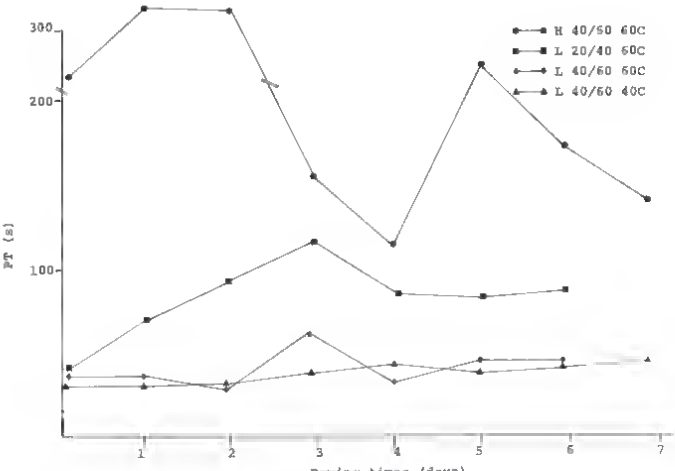


Fig. 21 FT versus drying times of various WR sands.

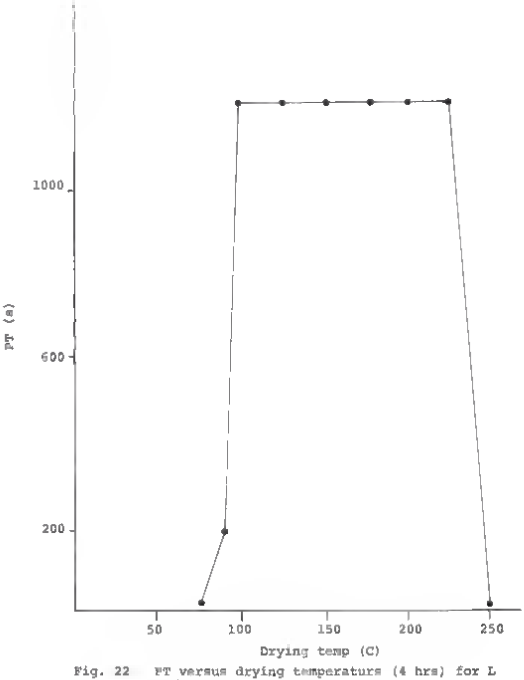


Fig. 22 PT versus drying temperature (4 hrs) for L 40/60 sands

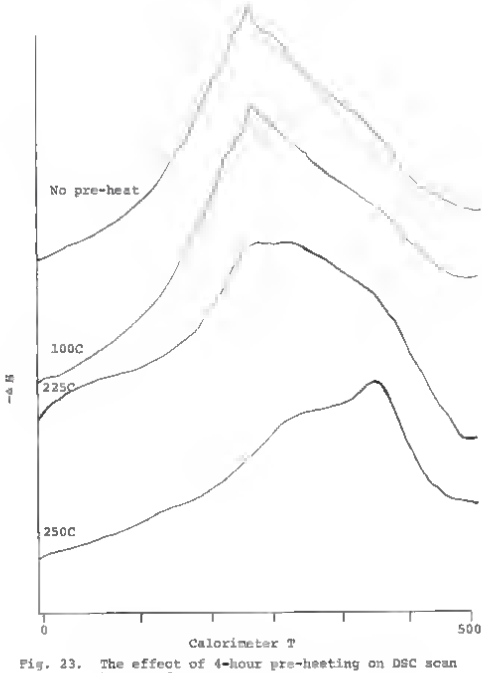


Fig. 23. The effect of 4-hour pre-heating on DSC scan WR L sand.

is obtained. This further supports the idea that the WR material is not removed on extraction at neutrality, but that the grains are hydrated. Temperatures up to 70C are not sufficient to dehydrate them to regenerate WR, but 100C is sufficient.

The large increase in WR on drying at 100C is genexel for all L samples whether natural (unchanged from their field condition) extracted at neutrality, or ones whose repellency has declined in the laboratory.

The resilience of this high-temperature form of repellency is shown by the data in Table 21, which shows the effects of various drying temperatures upon L material extracted at various pHs and followed by a neutral wash of equal volume.

The heating was done in a calibrated furnace. In all cases the WR goes to zero between 225 and 250C. Thus, whatever the chemistry of extraction may be, this ultimate transition remains constant. It is not until after the pH = 13 extraction that high temperature fails to regenerate repellency. Even at the relatively low drying temperature of 100C, significant repellency is retained after extraction at pH = 12. That WR decreases somewhat supports the idea that the material is a weak acid.

One would like to know why, in the cases of the pH = 10 and 12 extractions, one loses significant repellency on 100C drying but not on 225C drying. Perhaps

Table 21. PTs of L sands at different extraction pHs and drying temperatures.

Extractn. pH	Dry temp. 75	100	200	225	250
	PT,s				
no extractn.	40	>1200	>1200	>1200	0
7.5	-	>1200	-	>1200	0
10.0	15	328	-	>1200	0
12.0	4	64	-	>1200	0
13.0	0	0	-	0	0

new WR material is being created at this temperature. Is this situation parallel to fire-induced repellency? At this time there are no answers to these questions.

Table 21 also serves to provide evidence against the Wander (1949) mechanism. It was found that basic extraction of calcium stearate coated sand grains had no ill-effect upon the PT. The extraction was done with one-molar NaOH at a 10:1 volume to weight-of-sand ratio. In the case of the L sand, all repellency had vanished at all temperatures with a pH = 13 extraction.

The precipitous decline of all PT to zero on 250C drying in Table 19, led to the following experiments. L material was dried in a calibrated furnace, again for 4 hours. The curve in Fig. 22 shows that little happened until 90C, at which temperature there was a slight increase in PT. At 100C this increased to >1200s. The PT remained above 1200s until 250C, where it dropped to zero. The increase near 100C suggests a drying phenomenon. The precipitous drop at 250C indicates that the organic basis for WR is changing in some dramatic way.

To test the latter idea, samples of 40/60 L material which had been pre-treated at various temperatures were scanned from zero to 500C on a differential scanning calorimeter (DSC). Figure 23 shows the results. Until the 250C pre-treatment is reached, the oxidation patterns of the various samples are not very different from one

another. This shows that the oxidizeable organics are not changing greatly with temperature of pre-treatment. This is consistent with the rise in PT at near 100C being due to dehydration, not a change in the structure of the organic matter on the sand. After the 250C pre-treatment, the oxidation pattern changes dramatically. The oxidation peaks become much less symmetrical and are shifted to the right.

The DSC patterns do not indicate that a great amount of organic material is lost from the sand in the 250C treatment. The area under this oxidation curve is not much less than under the others. This is consistent with the fact that the sand after the 250C pre-treatment is still grey in color, not white, as is the sample after oxidation at 500C in the calorimeter. What is different is the pattern of oxidation, indicating that the 250C pre-treatment has caused a profound change in the molecular organization of the surface organics.

Extractions with Organic Solvents

The L material was extracted with several organic solvents in the same manner as water extractions. The solvents were dried over zeolites prior to use.

The ethanol result (Table 22) is interesting because its most likely effect is that of drying the sand surface. Thus, it acts like 100C drying, but less dramatically. Hexanes have a similar effect for unknown reasons.

Table 22. PT effects of various solvents on L (40/50) sands.

Solvent	Water		Methanol		Acetonitrile		Ethanol		n-hexane	
Diel. const.	80.4		32.6		36.2		24.3		1.89	
Rep.	PT_i	PT_f	PT_i	PT_f	PT_i	PT_f	PT_i	PT_f	PT_i	PT_f
1	179	25	116	8	82	34	185	321	179	842
2	415	15	1200	250	82	23	10	20	415	1200
3	238	9	169	7	-	-	338	573	-	-
4	1200	28	-	-	-	-	-	-	-	-

The more polar solvents shown in Table 22 have the opposite effect. Like water, they reduce repellency. The methanol extraction gives us yet another chance to test the Wander hypothesis that methanol soluble Ca and Mg salts are responsible for WR. Wender claimed that a spot test on the methanol extract gave a strong Ca spot test. If this were the case in the L material, modern atomic absorption analysis should give a very strong reading for this ion. In fact, it showed our extract contained only 60 and 70 ppb of Ca and 45 and 42 ppb of Mg for two repititions of the extraction. Again, the conditions for Wander-type repellency are not met by our samples.

In a final experiment the methanol extracts from 20 gm L materials were re-applied to 3 gm of ignited sand. When the methanol had evaporated, the samples were dried at 70 and 100C and tested for FT. None was found. We conclude that while methanol certainly reduces FTs, whatever dissolves in it does not re-induce the phenomenon

CONCLUSIONS

The Occurrence of Water-Repellency Under Field Conditions

The results from the 100 RH experiments confirm that WR develops in the drying period following wetting (Bond, 1969; Holzhey, 1969). When a sand is wetted and then begins to dry, it is temporarily very near 100 RH. This is the optimum humidity for WR development. The results also support the contention that when a sand is wetted it is NWR (Jamison, 1945; Roberts and Carbon, 1971; Wilkinson and Miller, 1978). They further confirm the view of Holzhey (1969) that, during long periods of desiccation, WR declines rather than increases. The opposing view is held by Roberts and Carbon (1971).

A model for the variation of WR may be written as follows. Assume that we start with a previously WR sand which is thoroughly wet. It is NWR and remains so until the soil moisture declines to 100 RH. When this condition is reached, WR begins to develop due to microbial activity. The time-course experiments indicate that WR will grow so long as the soil is at about 100 RH. As soil moisture continues to decline, WR increase eventually stops. This occurs around 97% RH.

If soil moisture remains low for long periods of time, the degree of repellency will gradually decline via a

non-biological mechanism. Likewise, if a rain falls which is sufficient to wet the WR areas, the WR will immediately disappear. The results indicate that at temperatures attainable in the field, WR does not return by simple drying, but only on the reattainment of the 100 RH condition.

This model for WR development may not be universal. In particular, the situation as described by Roberts and Carbon (1971, 1972) may well occur in some places. However, the fact that all of the diverse WR materials tested here increased greatly in repellency at 100 RH and lost their repellency both on wetting and on long standing at low humidity, suggesting that these mechanisms are widespread. That WR produced at 100 RH in the laboratory declined in repellency in the same ways as did the natural samples, further supports the conclusion that this mechanism is of major importance.

The Microbiology of Water-Repellency

The organisms which correlate best with WR increase are the actinomycates. This is most clearly seen in the dilution experiments which show that, in the L sand, fungal growth is about the same at 90 RH (where little WR is generated) as at 100 RH, where it is quite strong. The bacteria actually do better at 90 RH, which makes them unlikely as WR generators. Actinomycete growth parallels the increase in FT quite closely.

Scanning electron microscopy supports this view, revealing that actinomycetes dominate the WR sand surface. Antibiotic work confirms that prokaryotic organisms are vitally involved in the development of WR in the L sand.

Two cautions are required here. First, while the preceding model seems to hold for the L sand, this is certainly not the only way WR can develop. We have seen that B sand PTs grew quite significantly under anaerobic conditions, which do not favor actinomycete proliferation. Second, it has to be remembered that correlation is not cause. While actinomycetes seem to correlate best with aerobic WR increase in the L material, it could still be that a particular fungus or bacterium is the true cause of the effect, but that its relatively small numbers are masked in the dilution experiment and hidden from the SEM field of view by the organic material.

Why do some sandy soils not become WR? The results indicate that in one case at least, that of Eustis fine sand, the phenomenon is in part biological. The E sand certainly contains some organism which suppresses WR increase in the L sand. Presumably the same suppressive power applies to the E sand itself.

Our inoculation experiments with organisms isolated from WR sands were not successful in generating significant PTs at naturally attainable temperatures. No inoculations maintained in saturated conditions produced

repellency at any drying temperature. However, when one particular actinomycete was maintained at 100 RH on ignited sand, significant repellency was produced when the sand was dried at 100C.

Scanning EM of the inoculated sands shows a high concentration of spores on the surfaces.

Natural WR does not appear to be due directly to the presence of hyphae, spores, or individual cells. Scanning EM of naturally WR materials shows no such structures as is the case with WR sands generated at 100 RH, unless they are specially handled.

The Chemical Basis of Water-Repellency

It was not possible to confirm the view of Wander (1949) and Roberts and Carbon (1972) that WR is the direct result of an organic coating over most of the sand grain surface. The experiments do not indicate that no coating exists, but only that if it exists, it is of great thinness.

The WR material does not appear to be removed from the sand when the sand is wetted. A large volume extraction of the sand had about the same effect on PT as did a single drop of water. Since drying at field-attainable temperatures does not re-induce WR, what becomes of the old WR material when WR is regenerated at 100 RH? It could be that the old WR material is simply sacrificed as food for the new microbial proliferation.

The aqueous solvent work confirms the accepted view that WR is base-sensitive. On high pH extraction, all existing and potential repellency is lost. Organic solvents tend to affect WR in inverse proportion to their dielectric constant. The chemistry of this is not understood.

LITERATURE CITED

Adhidari, M., and G. Chakrabarti. 1976. Contribution of natural and microbial humic acids to water-repellency in soil. J. Ind. Soil Sci. 24: 217-219.

Bond, R. D. 1969. Factors responsible for water-repellence of soil. In L. F. DeBano and J. Letey (eds.) Water-repellent soils. University of California, Riverside.

Bond, R. D. 1972. Germination and yield of barley when grown in a water-repellent sand. Agron. J. 64: 402-403.

Bond, R. D., and J. R. Harris. 1963. The influence of the microflora on physical properties of soil. I. Effects associated with filamentous algae and fungi. Aust. J. Soil Res. 2:111-122.

Bornemisza, E. 1964. Wettability of soils in relation to their physico-chemical properties. Ph.D. dissertation, University of Florida, Gainesville.

Brady, N. C. 1974. The nature and properties of soils. MacMillan, New York.

Brock, T. D. 1979. The biology of microorganisms. Prentice-Hall, Englewood Cliffs, New Jersey.

Chen, Y., and M. Schnitzer. 1978. The surface tension of aqueous solutions of soil humic substances. Soil Sci. 125:7-15.

Emerson, W. W., and R. D. Bond. 1963. The rate of water entry into dry sand and calculation of the advancing contact angle. Aust. J. Soil Res. 1:9-16.

Guzer, V. S., N. G. Bondarenki, and B. A. Byzov. 1982. A method for the direct study of the microbiotical soil state by the structure of the initiated microbial community. Pedobiologia 24:65-79.

Hayes, M. B. H., and R. S. Swift. 1978. The chemistry of soil organic colloids. In D. H. Greenland and M. H. B. Hayes (eds.) The chemistry of soil constituents. Wiley, New York.

Holzhey, C. S. 1969. Water-repellent soils in southern California. In L. F. DeBano and J. Letey (eds.) Water-repellent soils. University of California, Riverside.

Jamison, V. C. 1945. The penetration of irrigation and rain water into sandy soils of central Florida. Soil Sci. Soc. Proc. 9:25-29.

Jamison, V. C. 1946. Resistance to wetting in the surface sandy soils under citrus trees in central Florida and its effects upon penetration and the efficiency of irrigation. Soil Sci. Proc. 10:103-109.

Johnson, L. F., and E. A. Curl. 1972. Methods for research on the ecology of soil-borne plant pathogens. Burgess Publ. Co. Minneapolis, Minnesota.

King, P. M. 1981. Comparison of methods for measuring severity of water-repellence of sandy soil and assessment of some factors that affect its measurement. Aust. J. Soil Res. 19:275-285.

Kononova, M. M. 1961. Soil organic matter. Its nature, its role in soil formation and soil fertility. Pergamon Press, London.

Letey, J. 1969. Measurement of contact angle, water drop penetration time as critical surface tension. In L. F. DeBano and J. Letey (eds.) Water-repellent soils. University of California, Riverside.

Lindsay, W. L. 1979. Chemical equilibria in soils. Wiley, New York.

McGhie, D. A., and A. M. Posner. 1980. Water-repellence of a heavy-textured western Australian surface soil. Aust. J. Soil Res. 18:309-323.

Menzel, D. A., and R. S. Zscarro. 1964. The measurement of dissolved organic and particulate carbon in sea water. Limnol. and Oceanog. 9:138-142.

Meynell, G. G., and E. Meynell. 1965. Theory and practice in experimental microbiology. Cambridge University Press, England.

Miller, R. H., and J. F. Wilkinson. 1977. Nature of the organic coating on grains of non-wettable golf greens. Soil Sci. Soc. Am. J. 4:1203-1204.

Osborn, J. F. 1969. Effect of wetting agents and water on the germination and establishment of grass. In L. F. DeBano and J. Letey (eds.) Water-repellent soils. University of California, Riverside.

Pestka, S. 1975. Chloramephenicol. In J. W. Corcoran and F. E. Hahn (eds.) Antibiotics, III. Springer-Verlag, Heidelberg.

Pramer, D., and E. L. Schmidt. 1964. Experimental soil microbiology. Burgess Publ. Co., Minneapolis, Minnesota.

Reeder, C. J., and M. F. Jurgensen. 1979. Fire-induced water-repellency in forest soils of upper Michigan. Can. J. For. Res. 9:369-373.

Roberts, F. J., and E. A. Carbon. 1971. Water-repellence in sandy soils of southwestern Australia. I. Some studies related to field occurrence. Fld. Stn. Rec. Div. Fl. Ind. SCIRO (Austr.). 10.13-20.

Roberts, F. J., and E. A. Carbon. 1972. Water-repellence in sandy soils of southwestern Australia. Aust. J. Soil Res. 10:35-42.

Savage, S. M., J. P. Martin, and J. Letey. 1969a. Contribution of humic acid and a polysaccharide to water-repellency in sand and soil. Soil Sci. Soc. Am. Proc. 33:149-150.

Savage, S. M., J. P. Martin, and J. Letey. 1969b. Contribution of some soil fungi to natural and heat-induced water-repellency in sand. Soil Sci. Soc. Am. Proc. 33:405-409.

Schlessing, D., and G. Medoff. 1975. Streptomycin, dihydrostreptomycin and the gentamycins. In J. W. Corcoran and F. W. Hahn (eds.) Antibiotics, III. Springer-Verlag, Heidelberg.

Stevenson, F. J., and R. M. Goh. 1971. Infrared spectra of humic acids and related substances. Geochim. and Cosmochim. Acta. 35:471-483.

Tiedje, J. M., and F. B. Dazzo. 1982. Soil microbiology. In V. S. Kilmer (ed.) Handbook of soils and climate in agriculture. CRC Press, Inc., Boca Raton, Florida.

Volk, B. G., and M. Schnitzer. 1973. Chemical and spectroscopic methods for assessing subsidence in Florida histosols. Soil Sci. Soc. Am. Proc. 37:886-888.

Waksman, S. 1936. Humus. Williams and Wilkins Co., Baltimore, Maryland.

Wander, I. W. 1949. An interpretation of the cause of water-repellent sandy soils found in citrus groves of central Florida. Science. 110:299-300.

Whittig, L. D. 1965. X-ray diffraction techniques for mineral identification and composition. In C. A. Black, E. D. Evans, J. L. White, L. E. Ensminger, and F. E. Clark (eds.) Methods of soil analysis. Agronomy Monographs #9, Madison, Wisconsin.

Wilkinson, J. F., and R. H. Miller, 1978. Investigation and treatment of localized dry spots on sand golf greens. Agron. J. 70:299-304.

Williams, S. T., and F. L. Davies. 1965. Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. J. Gen. Microbiol. 38: 25-261.

Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology. 41:232-237.

Yuan, T. L., and L. C. Hammond. 1968. Evaluation of available methods for soil wettability with particular reference to soil-water contact angle determination. Soil and Crop Sci. Soc. Fla. Proc. 28: 56-63.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David H. Hubbell, Chairman
Professor of Soil Science

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